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Chemical Warfare Agents: Estimating Oral Reference Doses

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Contents

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I. Introduction

The FY 1993 Defense Authorization Act [Public Law (PL) 102–484, Sect. 176] directed the U.S. Department of the Army (DA)[1] to examine the scale of effort and consider plans needed to safely dispose of nonstockpile chemical materiel (NSCM), previously identified as an area of national concern in House Appropriations Report 101–822 from the FY 1991 Defense Appropriations Act. Nonstockpile chemical materiel is defined in the Appropriations Report as "... lethal wastes from past disposal efforts, unserviceable munitions, chemically contaminated containers, chemical production facilities, subsequently located chemical munitions, sites known to contain significant concentrations of buried

[1]Abbreviations and acronyms used in this review are listed in Appendix A.

chemical weapons and waste, and binary weapons and components." Items considered NSCM are further characterized as chemical materiel outside of the U.S. retaliatory stockpile of lethal chemical agents and munitions (described more fully in Carnes and Watson 1989; DA 1988).

The NSCM have been the subject of recent surveys and reports prepared by the U.S. Army Chemical Materiel Destruction Agency (USACMDA) in partial fulfillment of directives contained in PL 102–484 (USACMDA 1993a,b). An idea of the scale of the geographic area of interest for chemical agents can be gained by examination of Table 1. Inventories and site characterization from records searched describe each of 82 NSCM locations in 33 states, the Virgin Islands, and the District of Columbia for the U.S. locations (USACMDA 1993b). The USACMDA reports also address documentation requirements that the United States must meet as a signatory of the *Convention on the Prohibition of the Development, Production, Stockpiling and Use of the Chemical Weapons and on Their Destruction* (Chemical Weapons Convention, or CWC). Additional classified text lists potential overseas burial sites (see Appendix D in USACMDA 1993b).

The majority of historical disposal techniques used were burials, although some NSCM were intentionally placed in water bodies. There is potential for soil and groundwater contamination at sites described in the inventory.

A. Objectives

The U.S. Army Environmental Center (USAEC) has responsibilities for supporting installation restoration (environmental cleanup) activities at Army installations and property nationwide. USAEC functions as the program manager for the Army's Installation Restoration Program (IRP). In recent years, the need for decision criteria to determine the scale and level of installation restoration required at sites that may include chemical warfare agent contamination, at both active installations and formerly used defense sites (FUDS), has become increasingly evident. However, key data pertaining to the chronic toxicity of these substances, necessary for performing the risk analyses that normally are part of the cleanup decision process, have not been readily available. The purpose of the investigation discussed in this review was to examine the existing toxicological literature and, where possible, to develop chronic reference doses[2] for the chemical agents of primary interest. This provision would enable the associated cleanup process to proceed in a responsible and efficient manner toward the ultimate goal of public and environmental health protection.

B. Approach

Decision criteria for determining acceptable cleanup goals at sites contaminated with chemical agents require evaluation of the potential health risks associated with residual amounts of chemical agents in various environmental media. For

[2]Definitions of technical terms are provided in the Glossary in Appendix B.

Table 1. Summary of chemical materiel thought to be located at nonstockpile sites.

State—EPA region	Site	Materiel of concern[a]
Alabama—IV	Anniston Army Depot	GB, VX
	Ft. McClellan	GB, VX, mustard, HD, CK, CG, BZ, CX, AC
	Camp Sibert	? Mustard degradation products
	Huntsville Arsenal	Mustard
	Redstone Arsenal	HD, L, uncharacterized rounds, GB, VX
	Theodore Naval Ammunition Magazine	Mustard and/or its degradation products
Alaska—X	Adak	Mustard, L
	Chicagof Harbor	Mustard, L
	Gerstle River Test Center	Mustard, L, GB, GA, VX
	Unalaska Island	CAIS[b] vials
	Ft. Wainwright	CAIS[b]
Arizona—IX	Navajo Depot Activity	Mustard, white phosphorus, PWP
	Yuma Proving Ground	Mustard, GB, VX
Arkansas—VI	Ft. Chaffee	CAIS[b] residue
	Pine Bluff Arsenal	Mustard, HN, L, and degradation products, CAIS[b]
California—IX	Ft. Ord	Mustard, CAIS[b]
	Santa Rosa Army Airfield	CAIS[b]
	Edwards AFB	Mustard, GB, phosgene, CK, HCN
Colorado—VIII	Rocky Mountain Arsenal	GB, mustard, CG, VX
	Pueblo Army Depot Activity	Mustard
District of Columbia—III	American University	L, adamsite
Florida—IV	Brooksville Army Air Base	Mustard
	Drew Field	Mustard, CAIS[b]
	MacDill AFB	Mustard
	Withlacoochee	Mustard (Levinstein)
	Dry Tortuga Keys	Mustard
	Zephyr Hills Gunner Range	Mustard
Georgia—IV	Ft. Gillem	Mustard
	Ft. Benning	? G-agents ?
	Manchester	Mustard
Hawaii—IX	Kipapa Ammunition Storage Site	Mustard

Table 1. (*Continued*)

State—EPA region	Site	Materiel of concern[a]
	Schofield Barracks	H, L, CK, HCN, and residues, CAIS[b]
	Waiakea Forest Reserve	GB, BZ
Idaho—X	Targhee National Forest	Phosgene, NO_2
Illinois—V	Savanna Army Depot Activity	Mustard and residue
Indiana—V	Camp Atterbury	Mustard, CAIS[b]
	Naval Weapons Support Center	Mustard, CAIS[b]
	Newport Army Ammunition Plant	VX and residue
Kansas—VII	Marysville	Mustard
Kentucky—IV	Blue Grass Army Depot	Mustard
Louisiana—VI	England AFB	CAIS[b], phosgene
	Ft. Polk	CAIS[b] (mustard, L)
	Mississippi R. near New Orleans	Bombs with unknown fill
	Concord Spur	Mustard
Maryland—III	Edgewood Area-APG	VX, mustard, GA, GB, white phosphorus, riot control agents; spectrum of US, foreign, and experimental CW
Mississippi—IV	Columbus Army Airfield	Mustard
	Horne Island	Mustard, arsenic-containing agents, unspecified others
	Camp Shelby	Mustard
Nebraska—VII	Nebraska Ordnance Plant	Mustard
Nevada—IX	Hawthorne Army Amm. Plant	Mustard, phosgene, unspecified others
New Jersey—II	Lakehurst Naval Air Base	Unspecified "toxic agent shells"
	Raritan Arsenal	Mustard and residues
	Delaware Ordnance Depot	Phosgene
	Ft. Hancock	Unspecified "gas storage cylinders"
New Mexico—VI	Wingate Ordnance Depot	Mustard
New York—II	Mitchel Field	CAIS[b]
North Carolina—IV	Camp LeJeune	CAIS[b], CN, unspecified others
	Laurinburg-Maxton Army Air Base	Mustard

Table 1. (*Continued*)

State—EPA region	Site	Materiel of concern[a]
Ohio—V	Ravenna Army Ammunition Plant	Mustard
Oregon—X	Umatilla Depot Activity	Mustard, VX, other "mixed contamination"
Pennsylvania—III	Defense District Region East (formerly New Cumberland Army)	CAIS[b]
South Carolina—IV	Charleston Army Depot	Mustard
	Naval Weapons Center	Mustard
South Dakota—VIII	Black Hills Ordnance Depot	Mustard, CG
Tennessee—IV	Defense Depot Memphis	Mustard, CAIS[b]
Texas—VI	San Jacinto Ordnance Depot	Phosgene, mustard
	Ft. Hood	Mustard, CN
	Camp Stanley Storage Activity	Mustard
	Camp Bullis	Mustard, CN, CS, phosgene, PS, white phosphorus
Utah—VIII	Dugway Proving Ground	VX, GA, GB, GD, CS, mustard, agent residues, foreign chemical munitions, unspecified others; biologicals
	Defense Depot Ogden	CAIS[b], mustard, phosgene, smoke bombs
	Tooele Army Depot	Mustard and residues, smoke pots, GA, incendiaries
Virginia—III	Ft. Belvoir	CAIS[b]
Washington—X	U.S. Naval Magazine	Phosgene
Virgin Islands—II	(Former) Ft. Segarra (St. Thomas, Water Island)	CG, CK, HCN, phosgene, H, HT, GA

[a]GA, GB, GD, and VX are organophosphate nerve agents with anticholinesterase properties; H, HD, and HT are various formulations of sulfur mustard (vesicant); HN is nitrogen mustard (vesicant); L is the organic arsenical vesicant, lewisite. The following are less common: adamsite is an organic arsenical vomiting agent; AC is hydrogen cyanide (HCN); BZ is 3-quinuclidinyl benzilate, a hallucinogen; CK is the casualty agent cyanogen chloride; CG is phosgene (carbonyl chloride), a choking agent; CX is phosgene oxime (vesicant); CN is chloroacetophenone ("tear gas") and is used as a riot control agent; CS is o-chlorobenzalmalononitrile ("tear gas") and is used as a riot control agent.

[b]Chemical Agent Identification Set, a training aid containing vials of various chemical warfare agents normally in dilute chloroform solution. See USACMDA (1993a,b) for more detailed explanation.

Sources: USACMDA (1993a,b).

any environmental contaminant, potential health risks are determined by comparing estimates of exposure during current or future use of the sites with some measure of the toxic potency of each of the individual contaminants. Using standard exposure assumptions for inhalation exposures, the DA has developed health-based criteria (exposure limits) for personnel in the workplace and on the battlefield (DA 1987, 1990a). In addition, the U.S. Public Health Service has developed agent exposure limits for inhalation exposures to civilians and workers (52 *FR* 48458; 53 *FR* 8504). These exposure limits are shown in Table 2. However, for soil or water contamination that might result in accidental ingestion or skin contact, there are no media-specific exposure limits for any chemical agent. This lack occurs because such exposure limits are highly dependent on the characteristics of the population exposed and on the frequency and duration of exposure (e.g., a drinking water control limit would vary depending on how much water is consumed each day and over what duration of time). Therefore, control limits for contaminated soil or water must be determined on a site-by-site basis. The key step in this process is a comparison of expected exposure levels with reference toxicity values, the standard approach used in Superfund risk assessments (USEPA 1989).

For assessing noncancer health risks, the relevant toxicity value for each contaminant is expressed as a reference dose (RfD), which is derived from experimental or epidemiological data. An RfD is the daily exposure level or dose (usually expressed in units of milligrams of chemical per kilogram body weight per day) for the human population, including sensitive subpopulations, that is likely to be without an appreciable risk of deleterious effects. RfDs can be calculated for a subchronic exposure duration (2 wk to 7 yr) or for a chronic exposure duration (7 yr to a lifetime). A daily exposure at or below the RfD is not likely to be associated with health risks, but as the amount of chemical that an individual is exposed to increases above the RfD, the probability that an adverse effect will occur also increases (Cicmanec et al. 1996). Unlike control

Table 2. Maximum control limits for agents in air as recommended by the Centers for Disease Control (Department of Health and Human Services).

Agent(s)	Workplace, 8-hr TWA (mg/m^3)[a]	General population, 72-hr TWA (mg/m^3)[a]
H/HD/HT[b]	3×10^{-3}	1×10^{-4}
GA/GB[b]	1×10^{-4}	3×10^{-6}
VX[c]	1×10^{-5}	3×10^{-6}
Lewisite[b]	3×10^{-3}	3×10^{-3}

[a]Time-weighted average.

[b]Values recommended by U.S. Surgeon General's Working Group December 22, 1987 (52FR 48458); final promulgation March 15, 1988 (53FR 8504).

[c]Values recommended by the U.S. Department of Health and Human Services to the Secretary of the Army in October 1987; final promulgation March 15, 1988 (53FR 8504).

limits, a reference dose is a fixed value that is applicable to any contaminated site. However, this key measure of toxic potency, unlike the case for common industrial pollutants, was generally not available in the case of chemical agents. The method for calculating RfDs is described in detail in Section IV, and the RfDs for the individual chemical agents are calculated in Sections V through XIV.

The procedure used in this review identifies existing reference doses for the compounds of concern, or derives such values [identified as an estimated RfD (RfD_e)] from the available toxicological literature. Although several of the chemical agents are known or suspect carcinogens, carcinogenic potency values (slope factors) are not derived here.

C. Chemical Agents of Concern

Table 3 lists the chemical agents that have been most frequently found at Department of Defense (DOD) sites located in the U.S. The table also includes a brief description of the principal toxic effects of each chemical. The compounds evaluated in the current study were selected on the basis of frequency with which they are likely to be found, as well as their toxicity and persistence. This assessment builds on earlier published approaches to develop control limits for chemical agents in soil and water (Kistner et al. 1992; Watson et al. 1992). The focus of these previous works was the unitary stockpile, and was thus limited to the sulfur mustard agents H, HD, and HT; the organic arsenical vesicant agent L (lewisite); and the nerve agents GA, GB, and VX. The present work expands the list of evaluated chemical warfare agents to include agent HN2 (nitrogen mustard), agent CK (cyanogen chloride), and the nerve agent GD. No binary agent components are assessed here. The chemical and physical properties of each compound are summarized in Section II. The toxicity and RfD_e derivations are included in Sections V–XIV. Environmental fate and ecological effects data on each of the chemicals are presented in Section XV.

D. Review Process

The RfD_es presented in this report have undergone extensive review. The original draft of the parent document (Opresko et al. 1994) was reviewed by Dr. T. Bucci, Integrated Services, White Hall, AR, and Dr. I.K. Ho, University of Mississippi Medical Center, Jackson, MS. Early drafts of the RfD_e derivations were evaluated by Drs. M. Dourson and S. Valasquez, Toxicology Excellence for Risk Assessment, Cincinnati, OH; and Dr. W. Hartley, Tulane Medical Center, New Orleans, LA. Staff members of the U.S. Army Environmental Center, U.S. Army Center for Health Promotion and Preventive Medicine, and the U.S. Army Edgewood Research, Development and Engineering Center, all of Aberdeen Proving Ground, MD, provided very useful information, suggestions, and critiques that have been duly considered in the preparation of this report.

After internal and external review, the RfD_es for the chemical warfare agents were presented before the Material/Chemical Risk Assessment (MCRA) Work-

Table 3. Incidence and description of selected chemical surety materiel suspect at nonstockpile sites.

Agent	Chemical name	Acute mode of action	Nonstockpile sites where presence suspect (*N*)[a]
Vesicants			
Mustard formulations			
H (sulfur mustard)	bis(2-Chloroethyl)sulfide	Lethal blister agent (vesicant)	55
HD (sulfur mustard)	bis(2-Chloroethyl)sulfide	Lethal blister agent (vesicant)	
HT (sulfur mustard)	60% HD and <40% agent T[b]	Lethal blister agent (vesicant)	
HN2 (nitrogen mustard)	bis(2-Chloroethyl)methylamine	Lethal blister agent (vesicant)	
Organic arsenical			
L (lewisite)	Dichloro(2-chlorovinyl)arsine	Lethal blister agent (vesicant)	7
Nerve agents			
GA (tabun)	*N,N*-dimethylphosphoramidocyanidate, ethyl ester	Lethal anticholinesterase	5
GB (sarin)	Methylphosphonofluoridate, isopropyl ester	Lethal anticholinesterase	10
VX	*S*-(2-diisopropylaminoethyl)methyl phosphonothioate, *O*-ethyl ester	Lethal anticholinesterase	9
Blood agents			
CK (cyanogen chloride)	cyanogen chloride	Casualty agent; inactivates cytochrome oxidase and prevents oxygen utilization by cells	5

[a]Does not include suspect incidence of Chemical Agent Identification Sets (CAIS). These sets were training aids containing vials of various chemical warfare agents. See USACMDA (1993 a,b) for more detailed explanation. Agent GD (soman), pinacolyl methyl phosphonofluoridate, is a lethal anticholinesterase and is known from only one stockpile site.

[b]bis-[2(2-Chloroethylthio)ethyl] ether.

Sources: USACMDA (1993a,b).

ing Group of the Environmental Risk Assessment Program (ERAP). ERAP is a component of the Strategic Environmental Research Development Program (SERDP), a multiagency [U.S. Environmental Protection Agency (USEPA), U.S. Department of Defense (DOD), and U.S. Department of Energy (DOE)] effort addressing agency-specific risk assessment needs. Submission of the RfD_cs to MCRA provided a mutually beneficial opportunity to have the proposed values reviewed, as well as allowing for a substantive MCRA/ERAP effort. MCRA has been developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values adopted by MCRA are to be submitted for consideration by EPA's Integrated Risk Information System (IRIS) Consensus Process. The MCRA Working Group consisted of Drs. Jim Cogliano (chair) and Harlal Choudhury (U.S. EPA); Dr. Bruce Briggs (Geo-Centers); Lt. Cmdr. Warren Jederberg and Dr. Robert L. Carpenter (U.S. Naval Medical Research Institute); Dr. Elizabeth Maull and Mr. John Hinz (U.S. Air Force Occupational and Environmental Health Directorate); Drs. Glenn Leach and Winnie Palmer (U.S. Army Center for Health Promotion and Preventive Medicine); and Drs. Robert Young and Po-Yung Lu (Oak Ridge National Laboratory). In several meetings held in July, August, and September of 1996, MCRA considered and adopted the RfD_c values as presented in this report. These values were then submitted to the U.S. Department of the Army, Office of the Surgeon General, for consideration as interim criteria for conducting risk assessments at Army sites. In a memorandum dated August 19, 1996, the Army Office of the Surgeon General endorsed the proposed RfD_cs as interim criteria pending a formal review by the Committee on Toxicology (COT) of the National Research Council. A subcommittee of the COT is currently reviewing the chemical agent RfD_cs.

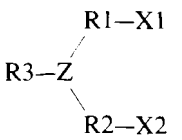
To all the individuals and organizations just mentioned in Section I, the authors would like to express sincere appreciation for their highly valued contributions.

II. Physical and Chemical Properties

The chemical agents evaluated in this review include the mustard agents HD (sulfur mustard), HT, and HN2 (nitrogen mustard); agent T, a thioether; the nerve agents VX, GA (tabun), GB (sarin), and GD (soman); the arsenic-based vesicant agent L (lewisite); and the blood agent CK (cyanogen chloride). A brief description of the physical and chemical properties of these compounds is given next.

A. Mustard Agents

There are two types of mustard agents, sulfur mustards and nitrogen mustards; the general structural formula of these compounds is as follows:

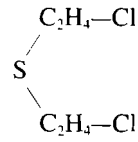


where:

Z = nitrogen or sulfur
X = halogen substituents
R = aliphatic groups, saturated, olefinic, or halogenated

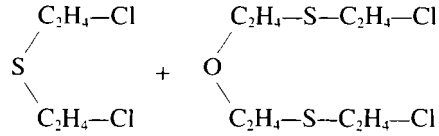
Agent HD is a sulfur-based mustard and agent HN2 is a nitrogen-based mustard. Agent HT is a mixture of agent HD and agent T, a thioether. The physical and chemical properties of HD, HT, and HN2 are summarized in Table 4. Mustards are oily liquids under ambient conditions and have a density greater than water. Agents HD and HN2 are very slightly soluble in water; agent HT is practically insoluble. Agents HD and HT are volatile under moderate temperatures; agent HN2 is less so. Both agents HD and HT are relatively stable at ambient temperatures. Agent HD undergoes hydrolysis, with the major degradation products being HCl and thiodiglycol (DA 1992a). For the environmental fate of these compounds, see Section XV.

Agent HD [bis(2-chloroethyl)sulfide; sulfur mustard; CAS no. 505-60-2] is a colorless, odorless, oily liquid with a molecular weight of 159.08 and a water solubility of 0.092 g per 100 g at 22 °C (DA 1974; MacNaughton and Brewer 1994) (see Table 4). Agent HD is also referred to as distilled mustard, with laboratory samples having a purity range of 95%–100% (DA 1974). Its chemical structure is



Commercial production of sulfur mustard results in the formation of a mixture containing about 70% bis(2-chloroethyl)sulfide and about 30% high molecular weight polysulfides. This mixture, referred to as agent H or Levinstein mustard, varies considerably in chemical composition and is not evaluated in this review.

Agent HT is a product of a reaction that yields about 60% agent HD [bis(2-chloroethyl)sulfide] and less than 40% agent T [bis-[2-(2-chloroethylthio)-ethyl] ether], plus a variety of sulfur contaminants and impurities. The composition of this mixture may change with time as the result of degradation reactions. The chemical structures of the two major components are



Agent T [bis-(2-(2-chloroethylthio)-ethyl) ether; CAS no. 63918–89-8], a component of agent HT, is added to lower the freezing point and increase the stability. Agent T is practically insoluble in water. The molecular formula is

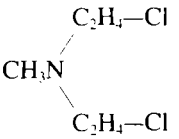
Table 4. Physical and chemical properties of mustard agents HD, HT, and HN2.

Property	HD	HT	HN2
Molecular formula	$C_4H_8Cl_2S$	~60% $C_4H_8Cl_2S$ <40% $C_8H_{16}Cl_2OS_2$	$C_5H_{11}Cl_2N$
Molecular weight	159.02	—	156.07
Physical state	Liquid	Liquid	Liquid
Color	Clear if pure, pale yellow to black if not	Clear to pale yellow	Clear to yellow
Boiling point (° C)	217	>228	87 (18 mmHg)
Specific gravity (water = 1)	1.27 (20° C)	1.265 (20° C)	1.118
Vapor pressure (mmHg, 25° C)	0.115	0.104	0.17
Vapor density (air = 1)	5.5	6.92	5.9
Volatility (mg/m^3, 25° C)	920	831	3.581
Viscosity (centistokes)	3.95 (20° C)	6.05 (20° C)	—
Water solubility (g/L)	0.92 (22° C); 0.68 (25° C)	Practically insoluble	Slightly soluble
Henry's law constant (H) ($atm \cdot m^3/mol$)	2.4×10^{-5}	—	—
Half-life in water (min)	8.5 (25° C); 5 (22° C)	—	—
Solubility in organic solvents	Soluble in fatty solvents and other common organic solvents	—	Ssoluble in many organic solvents and oils
Flash point (° C)	105	~100	—
Chemical stability	Stable for days to weeks	Stable at ambient temperatures	Unstable
Reactivity	Destroyed by strong oxidizing agents	—	Reacts rapidly with water
Log octanol–water partition coefficient	1.37	—	—

Sources: Clark (1989); DA (1988, 1990b, 1992a); FOA (1992); Harris et al. (1979); Papirmeister et al. (1991); Small (1984); Windholz et al. (1983).

$C_8H_{16}Cl_2OS_2$, with a molecular weight of 263.3 (DA 1974). Its chemical structure is shown above.

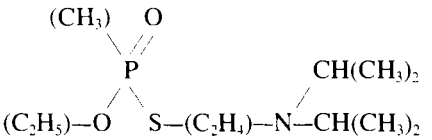
Agent HN2 [bis(2-chloroethyl)methylamine; mechlorethamine; nitrogen mustard; CAS no. 51–75-2] is a clear to yellow liquid (see Table 4). Its chemical structure is



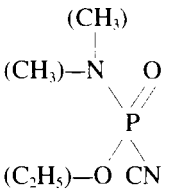
B. Nerve Agents

Four nerve agents are evaluated here; agents VX, GA (tabun), GB (sarin), and GD (soman). All are organophosphate compounds; VX has a sulfur substituent group, GA has a cyanide group, and agents GB and GD both have fluoride groups. Physical and chemical properties of agents VX, GA, GB, and GD are summarized in Table 5.

Agent VX (*O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothioate; CAS no. 50782–69-9) is a colorless to straw-colored liquid with a molecular weight of 267.37 (DA 1974; MacNaughton and Brewer 1994). Its chemical structure is



Agent GA (tabun; ethyl dimethylamidocyanophosphate; CAS no. 77–81-6) is a colorless to brown liquid with a molecular weight of 162.1 (DA 1974; MacNaughton and Brewer 1994). Its chemical structure is



Agent GB (sarin; isopropyl methylphosphonofluoridate, CAS no. 107–44-8) is a clear to straw-colored or amber liquid with a molecular weight of 140.1 (DA 1974; MacNaughton and Brewer 1994). Its chemical structure is

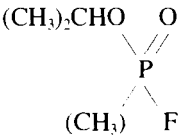


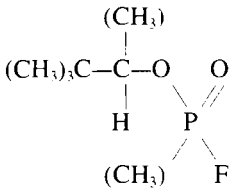
Table 5. Physical and chemical properties of nerve agents.

Property	Agent GA	Agent GB	Agent GD	Agent VX
Molecular formula	$C_5H_{11}N_2O_2P$	$C_4H_{10}FO_2P$	$C_7H_{16}FO_2P$	$C_{11}H_{26}NO_2PS$
Molecular weight	162.1	140.1	182.2	267.37
Physical state	Liquid	Liquid	Liquid	Oily liquid
Color	Clear to brown	Clear to straw-colored or amber	Clear	Clear to straw-colored
Boiling point (°C)	245	158	198	298
Density (g/mL, 25°C)	1.08	1.09	1.02	1.008
Vapor pressure (mmHg at 25°C)	0.07	2.9	0.40	0.0007
Volatility (mg/m^3, 25°C)	610	22,000	3,900	10.5
Vapor density (air = 1)	5.6	4.86	6.3	9.2
Flash point (°C)	78	>138	121	159
Viscosity (centistokes)	2.18 (25°C)	1.28 (25°C)	3.10 (25°C)	9.96 (25°C)
Water solubility (g/L)	98 (25°C); 72 (20°C)	Miscible	21 (20°C)	30 (25°C); 75 (15°C)
Rate of hydrolysis (half-life, hr, at pH 7)	8.5 (20°C)	39–41 (25°C)	80–83 (20°C)	400–1,000 (25°C)
Henry's law constant[a] (atm m^3/mol)	1.52×10^{-7}	5.34×10^{-7}	4.56×10^{-6}	8.19×10^{-9}
Log octanol–water partition coefficient	1.18	0.15	1.02	2.09

[a]Values for GA and GD calculated by ORNL from volatility and solubility data.

Sources: Clark (1989); DA (1974, 1988, 1992a); Britton and Grant (1988); Small (1984); Windholz et al. (1983).

Agent GD (soman, pinacolyl methylphosphonofluoridate; CAS no. 96–64-0) is a colorless liquid with a molecular weight of 182.2 (DA 1974; MacNaughton and Brewer 1994). Its chemical structure is



C. Agent L (Lewisite)

Agent L [lewisite, dichloro(2-chlorovinyl)arsine; CAS no. 541–25-3] is an organic arsenical known for its vesicant properties (Rosenblatt et al. 1975). It is an amber to dark-brown liquid with a molecular weight of 207.32, a vapor pressure of 0.58 mmHg at 25 °C, a liquid density of 1.89 g/cm^3 at 25 °C; its freezing point is −18 °C, its boiling point is 190 °C, and it is negligibly soluble in water (DA 1974). Lewisite may occur as a trans-isomer and as a cis-isomer. In aqueous solutions, the cis-isomer undergoes photoconversion to the trans-isomer (Clark 1989). In the presence of moisture, lewisite is rapidly converted to the stable but highly toxic lewisite oxide (2-chlorovinylarsenous oxide) (Cameron et al. 1946). The chemical structure of lewisite is

$$Cl{-}CH{=}CH{-}AsCl_2$$

D. Agent CK (Cyanogen Chloride)

Agent CK (cyanogen chloride; CAS no. 506-77-4) is a halogenated cyanide with the chemical formula of ClCN. It is designated by the U.S Army as a nonpersistent blood agent (Fedoroff and Sheffield 1962). The physical and chemical properties of CK are listed in Table 6. CK is a colorless liquid or gas with a melting point of −6 °C, a boiling point of 13.8 °C (Hartung 1994), and a vapor pressure of 1000 mmHg at 25 °C (DA 1974). It is soluble in water (25 cm^3/mL at 20 °C) and organic solvents (Hartung 1994). CK is highly volatile (2,600,000 mg/m^3 at 12.9 °C) (DA 1974; Jacobs 1942) and undergoes hydrolysis in water (Kononen 1988). Hydrolysis half-lives range from 1 min at 45 °C to 10 hr at 5 °C (Bailey and Bishop 1970). CK undergoes considerable hydrolysis at alkaline pH, forming cyanic acid (HOCN) and hydrochloric acid; the same products are formed at a slower rate at acidic and neutral pH values (Clark 1989). Because of its extreme volatility and relatively rapid hydrolysis in water, the chemical is not expected to persist in surface waters.

III. Signs, Symptoms, and Mechanisms of Toxicity

A. Mustard Agents

1. Signs and Symptoms of Toxicity. Mustard vesicants (agents HD and HN2) are acutely toxic by direct contact, the skin, eyes, and upper respiratory tract

Table 6. Physical and chemical properties of agent CK (cyanogen chloride).

Synonyms	Chlorine cyanide; chlorocyanogen; CK
CAS Reg. No.	506-77-4
Molecular weight	61.48
Chemical formula	ClCN
Physical state	Colorless liquid or gas
Vapor pressure	1,010 mmHg at 20° C
Liquid density	1.218 at 4/4° C
Gas density	1.186 at 20/4° C
Melting point	−6° C
Boiling point	13.8° C
Vapor density	2 (air = 1)
Volatility	2,600,000 mg/m^3 at 12.9° C
Solubility	At 20° C, soluble in water (2,500 cm^3/100 mL); ethyl alcohol (10,000 cm^3/100 mL); and ether (5,000 cm^3/100 mL); soluble in all organic solvents, but tends to polymerize to cyanuryl chloride ($CNCl_3$) on storage
Flash point	Does not flash
Odor	Pungent; detectable at 1 ppm
Reactivity	With water, very slow; rapid at alkaline pH
Conversion factors	1 mg/L = 398 ppm; 1 ppm = 2.51 mg/m^3 at 25° C, 760 mmHg; 1 mg/m^3 = 0.4 ppm (ppm = volume/volume basis)

Sources: ACGIH (1991); Aldridge and Evans (1946); Budavari et al. (1989, 1996); Hartung (1994); Jacobs (1942); Lide (1991); Weiss (1980); WHO (1970).

being the primary target organs (Sidell and Hurst 1992). Exposure to the skin can result in erythema, itching, burning pain, edema, blister formation, ulceration, and necrosis. Inhalation exposures produce inflammation and necrosis of the epithelium of the nose, pharynx, larynx, trachea, and bronchi and result in cough, hoarseness, moist rhonchi and rales, nasal bleeding, and nasal discharge. Effects on the eyes include irritation, reddening, conjunctivitis, photophobia, blepharospasm, pain, and corneal damage. Other reported signs and symptoms of systemic toxicity include nausea, vomiting, fever, depression, and malaise (ITII 1975; Sidell and Hurst 1992). Systemic effects include damage to the hematopoietic system, resulting in leukopenia. Delayed effects that may occur following acute exposures include eye lesions, chronic bronchitis, and respiratory tract and skin cancers. Information on adverse effects following long-term exposures to concentrations that are less than acutely toxic is very limited. Health effects of mustard agents have recently been reviewed by the Agency for Toxic Subtances and Disease Registry (ATSDR 1992), the Institute of Medicine (1993), Sidell and Hurst (1992), Somani (1992), and Watson and Griffin (1992).

2. *Mechanisms of Toxicity.* The acute toxic effects of mustard vesicants are usually attributed to the consequences of alkylation reactions with organic com-

pounds, including nucleoproteins such as deoxyribonucleic acid (DNA). Alkylation reactions can result in physiological and metabolic disturbances as well as genotoxic effects. Several hypotheses have been advanced concerning the primary cause of cell death following acute exposures. As reviewed by Papirmeister et al. (1991), the three major hypotheses are as follows

Poly(ADP-ribose) polymerase (PADPRP) hypothesis: In this theory, DNA is the initial target of the mustard agent. Alkylated DNA purines undergo spontaneous and enzymatic depurination, leading to the production of apurinic sites that are cleaved by apurinic endonucleases to yield DNA breaks. Accumulation of DNA breaks leads to activation of the chromosomal enzyme PADPRP, which utilizes nicotinamide adenine dinucleotide (NAD^+) as a substrate to ADP-ribosylate and a variety of nuclear proteins, causing severe lowering of cellular NAD^+. Depletion of NAD^+ results in the inhibition of glycolysis, and stimulation of the nicotinamide adenine dinucleotide phosphate ($NADP^+$)-dependent hexose monophosphate shunt (HMS) pathway follows as a result of the accumulation of glucose-6-phosphate, a common precursor for both glycolysis and the HMS. Induction and secretion of proteases is stimulated as a result of enhanced HMS activity, and this leads to pathological changes in the cell.

Thiol-Ca^{2+} peroxidation hypothesis: The first step in this process is thought to be the alkylation of glutathione (GSH) by the mustard agent. Depletion of GSH subjects protein sulfhydryl groups to damage from the agent or from reactive cellular oxidants. Proteins most susceptible to damage include Ca^{2+} translocases (Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase), which are dependent on thiol groups to maintain cellular Ca^{2+} homeostasis, and microfilamentous proteins, in which loss of sulfhydryl groups could result in disruptions of the cytoskeletal and structural integrity of the plasma membrane.

Lipid peroxidation hypothesis: According to this hypothesis the mustard agent causes depletion of GSH which, in turn leads to the buildup of highly toxic oxidants, usually through H_2O_2-dependent reaction sequences. The oxidizing agents react with membrane phospholipids to form lipid peroxides, initiating a chain reaction of lipid peroxidation that can lead to alterations in membrane fluidity, loss of membrane protein function, and loss of membrane integrity.

Because of their alkylating and electrophilic properties, mustard agents change the structure of nucleic acids, cellular membranes, and proteins (Somani 1992). Bifunctional alkylation of the nitrogenous bases of DNA leads to cross-linking, which prevents normal replication processes. Thus, mustard agents interfere with normal DNA synthesis and cellular division and are likely to have greater effects on dividing cells than on normal cells (Somani 1992).

B. Nerve Agents

1. Signs and Symptoms of Toxicity. Nerve agents are toxic by all routes of exposure (DA 1974). Toxic effects, which can appear within seconds or minutes

following exposure, include the following: miosis, conjunctival congestion, eye pain, distorted vision, nasal discharge, salivation, excessive sweating, bronchoconstriction, increased bronchial secretions, cough, shortness of breath, nausea, vomiting, diarrhea, abdominal pain, muscle fasciculations, twitching, weakness, alterations in heart rate and blood pressure, loss of reflexes, slurred speech, ataxia, paralysis, loss of consciousness, convulsions, and coma (Sidell 1992; Somani et al. 1992). Forgetfulness, irritability, impaired judgment, decreased comprehension, a feeling of tenseness or uneasiness, depression, insomnia, nightmares, and difficulties with expression may result from low exposures (Sidell 1992). In animals, exposures to high concentrations cause convulsions which, if maintained for sufficiently long periods of time, result in irreversible brain damage (Somani et al. 1992). This damage may be a consequence of hypoxia (Sidell 1992). Myocardial degeneration and necrosis, as well as brain lesions, have also been observed in laboratory animals given convulsion-producing single doses of agent GB or GD (Singer et al. 1987). Death following acute exposures to nerve agents has been attributed to anoxia following respiratory paralysis, severe bronchoconstriction, and weakness or paralysis of the accessory respiratory muscles (Somani et al. 1992). Additional information on the toxicity of nerve agents can be found in review articles by Carnes and Watson (1989), Dacre (1984), Munro et al. (1994), Sidell (1992), Somani et al. (1992), and Watson et al. (1989b).

2. *Mechanisms of Toxicity.* Nerve agents are inhibitors of acetylcholinesterase (AChE), an enzyme responsible for deactivating the neurotransmitter acetylcholine (ACh) at some neuronal synapses and myoneural junctions. By a mechanism of phosphorylation or phosphonylation, nerve agents bind with the enzyme, thereby preventing deactivation of ACh (Somani et al. 1992). The buildup of ACh results in cholinergic effects in both the peripheral and central nervous system. Although the nerve agent-AChE complex can undergo a process of reactivation through spontaneous hydrolysis, it can also undergo an "aging" process (thought to be caused by a loss of an alkyl or alkoxy group), whereby it becomes resistant to reactivation. Studies reviewed by Sidell (1992) indicate that different nerve agents have different rates of reactivation and aging. The complex formed between agent VX and AChE does not age significantly, and the rate of spontaneous reactivation in humans can be 0.5%–1%/hr for the first 48 hr (Sidell and Groff 1974). In contrast, agent GD ages very rapidly, with a $t_{1/2}$ (time required for 50% of the enzyme to become resistant to reactivation) of 1.3 min (Harris et al. 1978). The aging half-time for agent GA is 46 hr, as calculated from a rate constant of 2.5×10^{-4}/min (deJong and Wolring 1978), and the $t_{1/2}$ for agent GB has been reported to be 5 hr (Sidell and Groff 1974). In the latter case, approximately 5% of the GB-enzyme complex reactivated spontaneously. In contrast to the results of these latter studies, Grob and Harvey (1958) had earlier reported that both GA and GB combined with ChE almost irreversibly within 1 hr when tested *in vitro*.

3. Effects on the Nervous System. The anticholinesterase effects of the organophosphate nerve agents can be characterized as being muscarinic, nicotinic, or central nervous system (CNS) related. Muscarinic effects occur in the parasympathetic system and result in miosis, conjunctival congestion, ciliary spasm, nasal discharge, bronchoconstriction, increased bronchial secretion, anorexia, vomiting, abdominal cramps, diarrhea, sweating, salivation, bradycardia, and hypotension. Nicotinic effects occur in somatic (skeletal/motor) and sympathetic systems, resulting in muscular fasciculations and paralysis. Effects on the CNS may be manifested as confusion, loss of reflexes, slurred speech, anxiety, forgetfulness, irritability, impaired judgment, depression, insomnia, and fatigue (Sidell 1992; Somani et al. 1992).

Although the inhibition of AChE within neuroeffector junctions or the effector itself is thought to be responsible for the major toxic effects of organophosphate chemical agents, these compounds can apparently affect nerve impulse transmission by more direct processes as well. In addition to AChE inhibition, VX reacts directly with ACh receptors and receptors of other neurotransmitters (e.g., norepinephrine, dopamine, gamma-aminobutyric acid) (Bakry et al. 1988; Chen and Chi 1986; Churchill et al. 1985; Ho and Hoskins 1983; Idriss et al. 1986; Zhao et al. 1983). Bakry et al. (1988) reported that the direct action of nerve agents on nicotinic and muscarinic ACh receptors may occur when concentrations in the blood rise above micromolar levels, whereas at lower levels the action is mainly the result of inhibition of AChE; however, nanomolar blood concentrations of VX and soman may directly affect a small population of muscarinic ACh receptors that have a high affinity for [^{3}H]-*cis*-methyldioxalane binding. Agent VX may also counteract the effects of ACh by acting as an open channel blocker at the neuromuscular junction, thereby interrupting neuromuscular function (Albuquerque et al. 1985; Bakry et al. 1988).

4. Delayed Neuropathy. Exposure to some organophosphate ChE inhibitors results in delayed neurotoxic effects (distal neuropathy, ataxia, and paralysis) several days to several weeks after exposure. These effects, characterized by axon and myelin degeneration, are not associated with the inhibition of AChE, but rather with the inhibition (and subsequent aging) of an enzyme known as neuropathy target esterase (NTE). For some organophosphate compounds, delayed neuropathy can be induced in experimental animals at relatively low exposure levels, whereas for others the effect is only seen following exposure to supralethal doses when the animal is protected from acute cholinergic effects caused by ChE inhibition. In either case, there is evidence that a threshold exists below which delayed neuropathy does not occur. Studies reviewed by Somani et al. (1992) indicated that in chickens (a species particularly susceptible to this effect) a 70% decrease in brain NTE activity 24–48 hr after exposure is related empirically to the subsequent development of delayed neuropathy. According to Husain et al. (1995), a minimum of 45% NTE inhibition is needed to produce delayed neuropathy after multiple exposures. Agent VX does not inhibit NTE *in vitro* (Vranken et al. 1982), and there are no experimental data to indicate

that it causes delayed neuropathy *in vivo*, even at supralethal doses. Agents GB, GA, and GD inhibit NTE *in vitro* (Vranken et al. 1982). Although lethal doses of agents GB and GD did not inhibit NTE when tested *in vivo* in antidote-protected chickens (Crowell et al. 1989), supralethal doses of all three G-agents produced delayed neuropathy in chickens (Gordon et al. 1983; Willems et al. 1984). Signs indicative of delayed neuropathy have also been observed in chickens receiving sublethal subcutaneous doses of agent GB (Husain et al. 1995), and in mice exposed to GB vapors for 20 min/d for 10 d (Husain et al. 1993), but not in rats receiving daily doses of GB by gavage for 90 d (Bucci and Parker 1992; Bucci et al. 1991).

5. Effect on Blood Cholinesterases. AChE is a natural component of human blood, where it is found associated with red blood cells (RBC-AChE). RBC-AChE activity, and the activity of a second type of ChE found in blood plasma (butyrylcholinesterase, or plasma-ChE), have been used to monitor exposure to organophosphate compounds (pesticides and nerve agents). Both RBC-AChE and plasma-ChE have been used as bioindicators of potential toxic effects of organophosphate ChE inhibitors. There is some evidence that RBC-AChE is as sensitive as brain ChE to the effects of nerve agents. Grob and Harvey (1958) reported that the *in vitro* concentrations producing 50% depression of brain-ChE and RBC-AChE activity were the same in the case of GA (1.5×10^{-8} mol/L), and only slightly different (3×10^{-9} mol/L and 3.3×10^{-9} mol/L) in the case of GB. However, *in vivo* animal studies indicate a poor correlation between brain and RBC-AChE in cases of acute exposures (Jimmerson et al. 1989), and this is reflected in the fact that blood ChE activity may not always be correlated with exposure or with signs and symptoms of toxicity (Holmstedt 1959; Sidell 1992). In general, systemic effects following acute exposures are likely to occur in humans when RBC-AChE is inhibited by 75%–80%, i.e., to 20%–25% of normal levels (Sidell 1992). However, local ocular or respiratory effects may occur without any inhibition of blood ChE activity, and repeated exposures over a period of several days may result in a sudden appearance of clinical signs and symptoms because of cumulative effects (Grob and Harvey 1958).

Conversely, blood ChE activity can become very low without overt signs or symptoms during chronic exposures to low concentrations of organophosphates, either because RBC-ChE has a slower rate of recovery compared to tissue ChE or because neural tissue has a noncholinesterase-dependent recovery pathway (Grob and Harvey 1958). Sumerford et al. (1953) reported that some orchard workers exposed to organophosphate insecticides had RBC-AChE values as low as 13% of preexposure values without any clinical signs or symptoms of exposure. Animal studies have demonstrated that repeated exposures to low concentrations of organophosphate insecticides can also result in increased tolerance levels (Barnes 1954; Rider et al. 1952). Similarly, Sumerford et al. (1953) reported that some workers exposed to organophosphorus insecticides showed symptomatic improvement with continued exposure. Such adaptation may result from increased rates of formation of blood ChE, from increased rates of meta-

bolic detoxification, or from alterations in the functional state of cholinergic systems. Additional information on the development of tolerance to organophosphate ChE inhibitors can be found in a review paper by Hoskins and Ho (1992).

The blood cholinesterases may, to some degree, provide a protective effect by binding with some fraction of the anticholinesterase compound (Raveh et al. 1997; Somani et al. 1992). However, not all nerve agents bind equally well with all cholinesterases. In tests conducted on dogs, Holmstedt (1951) found that GA affected RBC- and plasma-ChE to a nearly equal degree. In contrast, VX preferentially inhibits RBC-AChE, 70% compared with about 20% inhibition of plasma-ChE (Sidell and Groff 1974).

Other enzymes in the blood can react with ChE inhibitors, thereby reducing the amount available for binding with AChE. Cohen et al. (1971) reported a phosphofluoridate fluorohydrolase (termed sarinase) in the blood plasma of man and rodents that enhances the hydrolysis of agent GB; however, the enzyme's activity is almost three times greater in rats than in humans. Rats also have aliesterases (carboxylesterases) in their plasma that are not present in human plasma, and these compounds can bind with organophosphates (Augustinsson 1959; Fonnum and Sterri 1981; Jokanović 1989). The G agents have a strong affinity for carboxylesterases (Jokanović 1989); however, according to Fonnum and Sterri (1981), VX has a quaternary ammonium group that prevents it from being a substrate for aliesterases. The strong specificity of agent VX to AChE may account, in part, for the fact that it is more acutely toxic than the G-agents.

6. Intra- and Interspecies Variation in Blood Cholinesterase Activity. Although blood ChE activity is used as a measure of exposure to organophosphate compounds, baseline activity levels can vary between individuals and between species. Sidell and Kaminskis (1975) reported that, for a test population of 22 human subjects, the highest coefficient of variation of RBC-ChE was 4.1% per single subject; the average range of variation was ±2.1% for men and ±3.1% for women. In individuals studied for 1 yr, the RBC-ChE activity varied by 11% in men and 16% in women. Yager et al. (1976) reported a 10.0% intraindividual coefficient of variation for RBC-ChE and 14.4% for plasma-ChE. Callaway et al. (1951) estimated that with only one preexposure measurement, the smallest measurable decrease was 15% of the baseline value for RBC-ChE activity and 20% of the baseline for plasma-ChE.

Data reviewed by Wills (1972) indicate that baseline plasma- and RBC-ChE activity levels of women may be lower than those of men. When compared to males, ChE activity levels of women, estimated from the mean values given in Wills (1972), ranged from 80%–83% in two studies to 94%–100% in three other studies. Woodward et al. (1994) reported that mean plasma- and RBC-ChE levels of female rhesus monkeys were 94% and 95%, respectively, those of males. There were no gender differences in the rates of reactivation of sarin (GB)-inhibited ChE or in aging of the enzyme; however, *de novo* regeneration of plasma-ChE was faster in male monkeys, while RBC-ChE regeneration was faster in females (Woodward et al. 1994).

A small subpopulation of men and women have genetically determined variants in their plasma ChE causing their plasma-ChE activity level to be very low (Harris and Whitaker 1962; Lehmann and Liddell 1969). Studies reviewed by Bonderman and Bonderman (1971) indicate that some homozygous individuals, constituting about 0.025% of the British population, may have plasma-ChE activity levels reduced to less than 25% of the normal value. Heterozygous individuals may have plasma-ChE activity levels 28%–114% of normal, with a mean of 64% (Lehmann and Liddell 1969). Individuals with these genetic variants may be unusually sensitive to some anticholinesterase compounds (Morgan 1989). In addition, plasma-ChE activity may be depressed in pregnant women and in individuals with liver disease, heart disease, allergic conditions, and neoplasms (Wills 1972).

Data compiled by Ellin (1981) reveal that the RBC-ChE activity for humans is slightly higher than that for monkeys and much higher than that for rats and other laboratory animals (Table 7). These differences in RBC-ChE activity may affect species sensitivity to a particular organophosphate compound. At the same time, the relative amount of plasma-ChE and other compounds in the blood (e.g., carboxylesterases and hydrolases) that can bind to and detoxify the organophosphate agents must also be considered. Rodents, but not humans, have high levels of carboxylesterases in the blood (and in the liver) and, as noted previously, these compounds may provide rats and mice with a higher level of resistance to anticholinesterase compounds to which they bind, such as GB, but not to others, such as VX (Augustinsson 1959; Fonnum and Sterri 1981; Fonnum et al. 1985; Jokanović 1989). As noted by Somani et al. (1992), interspecies variation in sensitivity to nerve agents (particularly the G-agents) may be ac-

Table 7. RBC-ChE activity in different species.

Species	RBC-ChE activity (μmol mL^{-1} min^{-1})	Optimum substrate concentration (M)[a]
Human	12.6	2×10^{-3}
Monkey	7.1	2×10^{-3}
Pig	4.7	1×10^{-3}
Goat	4.0	2×10^{-3}
Sheep	2.9	2×10^{-3}
Mouse	2.4	2×10^{-3}
Dog	2.0	2×10^{-2}
Guinea pig	2.7	2×10^{-3}
Rabbit	1.7	5×10^{-3}
Rat	1.7	5×10^{-3}
Cat	1.5	5×10^{-3}

RBC-ChE, red blood cell cholinesterase.

[a]Acetylthiocholine iodide concentration for maximum RBC-ChE activity.

Source: Ellin (1981).

counted for almost totally by carboxylesterase binding, not by species differences in hepatic or renal detoxification.

7. Potency of Nerve Agents as Cholinesterase Inhibitors. The potency of the anticholinesterase activity of nerve agents and other organophosphates is measured by either the bimolecular rate constant (k_i) for the reaction of the phosphate compound with the enzyme or by the molar concentration causing 50% inhibition of the enzyme (I_{50}) *in vitro*. The relationship between I_{50} and k_i as a function of time (t) is expressed by the following equation (Eto 1974):

$$I_{50} = \frac{0.693}{t \times k_i}$$

I_{50} data for several organophosphate nerve agents have been tabulated by Dacre (1984). The pI_{50} (negative log of the molar concentration causing 50% inhibition) was reported to be 8.8 for VX, 8.4–8.6 for GA, and 9.2 for GD (Dacre 1984; Holmstedt 1959). Grob and Harvey (1958) reported that the *in vitro* potency of GB ($I_{50} = 0.3 \times 10^{-8}$ mol/L) was five times that for GA ($I_{50} = 1.5 \times 10^{-8}$ mol/L).

Relative potency of nerve agents can also be expressed in terms of the *in vivo* dose necessary to produce the same level of ChE inhibition by a specific exposure route. Reported RBC-$AChE_{50}$ values for agent GB in humans are 0.003 mg/kg for an intravenous dose and 0.01 mg/kg for an oral dose (Grob and Harvey 1958). For agent VX, the RBC-$AChE_{50}$ is 0.001 mg/kg for an intravenous dose (Sidell and Groff 1974), 0.0023 mg/kg for an oral dose (Sidell and Groff 1974), and 0.029 mg/kg for a 24-hr dermal dose of an undiluted liquid (Sim and Stubbs 1960). The data for VX suggest that an oral dose about two times larger than an intravenous dose is needed to produce the same amount of RBC-AChE inhibition. Similar information is available from animal studies. Goldman et al. (1988) dosed Sprague-Dawley rats with 4 µg/kg VX by various routes of exposure and measured RBC-AChE activity after 3 and 24 hr. The intravenous and subcutaneous routes resulted in the greatest decreases in RBC-AChE. RBC-AChE levels (expressed as fraction of control values) were 0.14 ± 0.07 at 3 hr and 0.20 after 24 hr for the intravenous injection and 0.13 ± 0.07 at 3 hr and 0.20 after 24 hr for subcutaneous injection. In contrast, RBC-AChE levels after intragastric administration were 0.48 ± 0.14 of controls at 3 hr and 0.46 after 24 hr.

8. Blood Cholinesterase Inhibition as a Critical Toxic Effect. Because nerve agents function primarily as AChE inhibitors, and because changes in blood ChE are a measure of potential toxic effects, an exposure or dose level that results in no significant depression in blood ChE activity can be used to establish maximum acceptable exposure limits. The use of this endpoint is, however, complicated by two factors: (1) the natural variability in blood ChE activity levels of individuals and (2) the fact that, at low doses, blood ChE can be inhibited significantly without any clinical signs of toxicity.

In humans, 15% inhibition of RBC-AChE is generally considered to be the minimum change that can be observed with any statistical reliability (Callaway et al. 1951). Existing human response data (Marquis 1988) indicate that human RBC-AChE inhibition of as much as 20% is not associated with adverse clinical signs or symptoms and should be considered only as evidence of organophosphate exposure. This contention is supported by the USEPA (1995a), which reported scientific agreement that statistically significant inhibition of ChE in multiple organs and tissues accompanied by clinical effects constitutes a hazard; however, in the absence of clinical effects, such inhibition may not be of biological significance. It is generally agreed that inhibition of RBC- and plasma-ChE contributes to the overall hazard identification of ChE inhibiting agents by serving as biomarkers (USEPA 1995a). Animal data have shown that exposure to low doses of nerve agents for extended periods of time can result in low blood ChE activity levels without signs of toxicity. Bucci et al. (1992a) found no evidence of toxicity in rats dosed intraperitoneally for 30 d with agent GA (up to 112 $\mu g\ kg^{-1}\ d^{-1}$), even though RBC-AChE activity was reduced about 37% in females (relative to controls). In oral toxicity studies conducted on rats, Bucci and Parker (1992) found that gavage doses of 0.3 mg GB $kg^{-1}\ d^{-1}$ for 90 d caused nearly a 50% reduction in RBC-AChE activity without signs of toxicity. Likewise, Goldman et al. (1988) reported no signs of toxicity, but 78%–80% reduction in RBC-AChE activity, in Sprague-Dawley rats dosed subcutaneously with 1.0 μg VX $kg^{-1}\ d^{-1}$ for 30 d or more. In sheep dosed orally with VX (15 μg VX/d), whole blood ChE was reduced to 4%–5% of the normalized baseline values (during the last 3 wk of the dosing period) without any signs of toxicity (Rice et al. 1971).

Changes in blood ChE activity have been used by USEPA as a critical endpoint in the establishment of oral RfDs for organophosphate pesticides. For example, in the case of malathion (USEPA 1995b), the no-observed-effect level (NOEL) was identified as the highest oral dose level at which no significant change in RBC-AChE or plasma-ChE activity was recorded in five human volunteers who received the compound orally for 47 d (Moeller and Rider 1962). The next highest dose was associated with a depression of about 25% in both RBC-AChE and plasma ChE, but no clinical signs of toxicity. The EPA approach, also used for other organophosphate pesticides, is, therefore, to identify the lowest-effect level (LEL) as the dose at which statistically significant decreases in ChE levels (RBC-AChE, plasma-ChE, or brain-ChE) occur, and then to base an RfD on the dose level at which the change in ChE is not statistically significant. This approach is also used in this review so that the RfDs developed for the nerve agents will not be disproportionally different from those for organophosphate pesticides; however, it should be emphasized that these values may be overly conservative if based solely on blood ChE values because, as noted earlier, the endpoint evaluated may occur in the absence of clinical signs of toxicity. For these reasons, in evaluating the experimental data for the nerve agents, such an effect was considered to be a minimal lowest-observed-adverse-effect level (LOAEL). Furthermore, in evaluating individual sets of data, added

weight was given to those cases in which significant changes in blood ChE (RBC or plasma) occurred relative to both control and preexposure values and where there was also evidence of a dose–response relationship.

C. Agent L (Lewisite)

1. Signs and Symptoms of Toxicity. Lewisite is a vesicant and a systemic poison (DA 1974; USACHPPM 1996). It may be lethal following inhalation, ingestion, or dermal exposure. It produces an immediate and strong stinging pain on the skin, with itching and irritation, erythema within 30 min, and possible blistering after about 13 hr (DA 1974). It also causes immediate pain and blepharospasm on contact with the eye, followed by edema of the conjunctivae, inflammation of the iris, and corneal opacity which may lead to permanent blindness. Inhalation of lewisite results in immediate irritation of the respiratory tract, causing burning pain, reflex coughing, and tightness of the chest. If the exposure continues, severe pulmonary edema can result. If absorbed into the body, the vesicant may cause diarrhea, restlessness, weakness, subnormal temperature, low blood pressure, hemolytic anemia, shock, and death (DA 1974). The toxicology of lewisite has been reviewed by Goldman and Dacre (1989), Trammell (1992), and Watson and Griffin (1992).

2. Mechanisms of Toxicity. The toxicological effects of lewisite are ultimately caused by its interaction with thiol groups of biologically active proteins such as enzymes. The interaction with sulfhydryl groups of enzymes may result in inhibition of enzyme function by the formation of stable cyclic structures with arsenic (As^{3+}) as a result of the reaction of the arsenic with the sulfhydryl groups of organic compounds such as those occurring in dihydrolipoic acid and in reduced keratin (De Bruin 1976). Dihydrolipoic acid is a dithiol cofactor active in several important enzyme systems (required for cellular respiration) including alpha-ketoacid oxidases such as pyruvate oxidase, 2-oxoglutarate oxidase, and aldehyde dehydrogenase. Lewisite combines with the dihydrolipoic acid to form stable six-member ring structures (cyclic thioarsenite complexes), thereby inactivating the enzymes. Overall, the end result of these interactions and the ultimate mechanism of lewisite toxicity appear to be energy depletion which, in turn, results in cell death. Organochloroarsines, of which lewisite is an example, are also potent alkylating agents; this feature suggests carcinogenic potential.

D. Agent CK (Cyanogen Chloride)

1. Signs and Symptoms of Toxicity. Initial signs and symptoms of acute exposure to CK include intense irritation of the nose, throat, and eyes, with coughing, tightness in the chest, and lacrimation (DA 1974). Moderate exposure may cause dizziness, dyspnea, retching, and involuntary urination and defecation. At high doses these effects are followed by convulsions, unconsciousness, failing respiration, pulmonary edema, and death. Long-term exposures to low doses may

lead to dermatitis, loss of appetite, headache, and upper respiratory tract irritation (USACHPPM 1996).

2. Mechanism of Toxicity. A review of the available literature suggested that the mechanism of toxic action of CK in mammals is a combination of respiratory irritation and interference of cellular metabolism by the cyanide ion; the overall toxicity of CK appears to be a function of released free cyanide and the intact CK molecule. However, rapid conversion of CK to cyanide in humans would be expected.

Halogenated cyanides, including CK, are highly toxic and possess some of the same properties as hydrogen cyanide and its soluble salts. However, at low concentrations, the halogenated cyanides behave more like highly irritating vesicant gases, producing severe lacrimatory effects and both acute and delayed pulmonary irritation and pulmonary edema (Hartung 1994).

The cyanide ion is one of the most rapidly fatal poisons, with the central nervous system (CNS) as the target organ. It is a potent intracellular poison that acts within seconds of entering the circulation. The cyanide ion exerts its toxic effect by forming a complex with the ferric ion of mitochondrial cytochrome oxidase, the enzyme that catalyzes the terminal step in the electron transport chain, thereby preventing utilization of oxygen by cells. Because cytochrome oxidase occupies a central role in the utilization of oxygen in all cells, its inhibition leads to the disruption of cellular respiration. Although the CNS is the primary target organ, blockade of electron transport by cytochrome oxidase causes hypoxia in all tissues (ATSDR 1995; Hardy and Boylen 1983; Mitchell and Carroll 1989).

The principal metabolic pathway for the detoxification of cyanide involves conversion to thiocyanate via the enzyme rhodanese. This enzyme, while being widely distributed throughout the body, has the highest activity in the mammalian liver. Calabrese (1991) presented interspecies comparisons of the activity level of rhodanese in tissues of several species including the dog, rhesus monkey, rabbit, and rat. The data showed that rats exhibited the highest enzyme activity, while dogs exhibited the lowest enzyme activity, with monkeys having an intermediate level of enzyme activity (but closer to that of dogs than that of rats). Thus, dogs have a lower ability to detoxify cyanide than do rats or primates.

IV. Methodology for Deriving Oral Reference Doses

A Reference Dose (RfD) is "an estimate (with an uncertainty spanning perhaps an order of magnitude or greater) of a daily exposure level for the human population, including sensitive subpopulations, that is likely to be without an appreciable risk of deleterious effects" (USEPA 1989). Noncancer health risks at CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act of 1980) Superfund sites are assessed by comparing potential levels of exposure for specific environmental pathways with reference doses indepen-

dently derived from laboratory or epidemiological data [see Cicmanec et al. (1996) for review of guidelines for noncancer risk assessments].

The methods used here to derive oral reference doses for chemical warfare agents are based on the procedures outlined in EPA's "Risk Assessment Guidance for Superfund" (USEPA 1989), as well as in the "Methods for the Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry" (USEPA 1994a). RfDs for chemical warfare agents were calculated using the available toxicological data obtained by searching governmental and nongovernmental bibliographic data bases. Because the derived values have not been officially verified by EPA, they are identified here as estimated RfDs (RfD_e).

RfDs are developed primarily for chronic exposure periods. For humans, a chronic exposure is defined by USEPA as an exposure duration lasting between 7 yr (approximately 10% of a human lifetime) and a full lifetime (USEPA 1989). Chronic RfDs are reviewed and verified by an EPA intraagency workgroup and are made available, on-line, on the Integrated Risk Information System (IRIS; USEPA 1997). For specific exposure pathways and certain subpopulations, exposure periods of less than 7 yr may be used. For example, for ingestion of contaminated soil, EPA has identified children in the 1- to 6-year-old group as being the most susceptible (USEPA 1990a).

EPA also allows for the use of subchronic and developmental RfDs in specific cases where exposures are less than chronic. Subchronic RfDs are to be used for characterizing potential noncarcinogenic effects associated with short-term exposures lasting from 2 wk to 7 yr (USEPA 1989). Developmental RfDs are used to evaluate the potential effects on the developing fetus following a single exposure event. Subchronic RfDs accepted for use in the preparation of Superfund risk assessments are available in EPA's Health Effects Assessment Summary Table (HEAST; USEPA 1996a).

In cases in which potential exposures are less than 2 wk and exclude developmental effects, EPA allows for the use of the One-day and Ten-day Health Advisories issued by the EPA Office of Water (USEPA 1996b) as nonregulatory guidance values. The Health Advisories are derived in a manner similar to that used for Reference Doses.

The oral Reference Doses derived in this report (RfD_e) are intended for chronic exposure durations. Standard EPA procedures are used whereby animal or human toxicity data are used to identify a dose or exposure that corresponds to a no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL) (USEPA 1989). The NOAEL is the exposure level at which there are no statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects may be produced at this level, but they are not considered to be adverse if they do not result in functional impairment or pathological lesions that affect the performance of the whole organism or which reduce an organism's ability to cope with additional challenge (USEPA 1994a). The LOAEL is the lowest exposure level at which there are statistically or biologically signifi-

cant increases in frequency or severity of adverse effects between the exposed population and its appropriate control (USEPA 1994a).

The NOAEL or LOAEL is then adjusted by the application of a set of Uncertainty Factors (UFs) and a Modifying Factor (MF), as shown in the following formula:

$$RfD_e = \frac{\text{NOAEL (or LOAEL)}}{UF_H \times UF_A \times UF_S \times UF_L \times UF_D \times MF}$$

where:

NOAEL = No-observed-adverse-effect level, expressed as mg chemical per kg body weight per day

LOAEL = Lowest-observed-adverse-effect level, expressed as mg chemical per kg body weight per day

UF_H = Human to sensitive human; an Uncertainty Factor up to 10 to account for variation in the general population, and intended to protect sensitive subpopulations

UF_A = Animal to human; an Uncertainty Factor up to 10 to be used when extrapolating from animal data to humans and based on the assumption that humans are likely to be more sensitive than animals

UF_S = Subchronic to chronic; an Uncertainty Factor up to 10 to be used when extrapolating from a subchronic study to derive a chronic RfD_e

UF_L = LOAEL to NOAEL; an Uncertainty Factor up to 10 to be used when a suitable NOAEL is not available and when a LOAEL is used instead

UF_D = Incomplete to Complete data base; an Uncertainty Factor up to 10 to be used when the available data do not adequately address all possible adverse outcomes in humans

MF = Modifying Factor greater than 0 and less than or equal to ten to be used to reflect a qualitative professional assessment of additional uncertainties in the critical study and in the entire data base; the default value for the MF is 1.0

As noted by Cicmanec et al. (1996), the choice of the appropriate UF or MF reflects case-by-case judgment by experienced risk assessors, and the magnitude of any composite UF is dependent on professional judgment for the total uncertainty in all areas. The fewest levels of uncertainty occur if an RfD is derived from chronic exposure data for humans; however, in many cases the only available human data are from acute exposure studies and the only available chronic or subchronic data are from animal studies. Thus, the use of animal toxicity data, a period of exposure that is less than chronic, and the identification of a LOAEL and not a NOAEL add several levels of uncertainty to the derivation of the RfD_e. These uncertainties are addressed by the use of the Uncertainty Factors just listed. Based on empirical evidence, the standard default value for each of these UFs is 10 [see Cicmanec et al. (1996) for a discussion of the rationale for using UFs of 10]. In the case of UF_H, there is both empirical evidence derived from single-dose data, as well as data on human variability in

pharmacokinetic parameters to indicate that a factor of 10 would, under most circumstances, account for intraspecies variability in susceptibility to toxic compounds (USEPA 1994a). UF_A accounts for interspecies variability in pharmacokinetics and pharmacodynamics, with the default assumption that humans are likely to be more sensitive than the test species; however, for some compounds humans may be equally sensitive or even less sensitive than the test species, and this possibility must be taken into account when using UF_A.

A standard default of 10 is used for UF_S to estimate a chronic RfD from a subchronic study; however, a smaller UF_S may be acceptable if the exposure duration represents a significant portion of the test species lifetime (e.g., 1 yr in a rodent study) (Cicmanec et al. 1996), or if the critical effect is more dependent on concentration than duration of exposure and the critical effect does not increase in severity with time (USEPA 1994a). The standard default UF_L is also 10, meaning that the NOAEL is estimated to be one-tenth of the experimental LOAEL; however, the true NOAEL may be only slightly lower than the experimental LOAEL, and statistical analysis of the dose–response data might show this to be the case. In practice, the value of UF_L has also been based on the severity of the effect, with an effect such as liver necrosis warranting the use of a UF_L of 10 whereas one involving only alterations in liver enzymes (which may or may not be clearly defined as a sign of toxicity) warranting the use of a UF_L of less than 10. The default for the data base UF (UF_D) is also 10; however, the magnitude of this UF varies on a case-by-case basis and, according to EPA, "should be defined by the nature of the plausible or possible pathogenesis processes (i.e., defined according to possible mechanism[s] of action for the observed effect[s]" (USEPA 1994a). For example, the lack of reproductive toxicity studies for a chemical from a class of chemicals that are not known reproductive toxins would not be considered a significant data base deficiency; therefore, a UF_D of less than 10 may be acceptable.

The minimum data base requirement for deriving an RfD is a single well-conducted subchronic animal bioassay that evaluates a comprehensive array of endpoints (Cicmanec et al. 1996). Such an RfD, however, has a very low confidence level associated with it. High confidence in an oral RfD for a chemical can be achieved if the data base consists of at least two chronic oral toxicity studies in different species, one two-generation reproductive toxicity study, and two developmental toxicity studies in different species. Chronic studies are considered an integral part of a complete data base, particularly for those chemicals that have the potential to bioaccumulate or whose toxic effects may accumulate over time. Conversely, there are chemicals that pose only an acute health hazard because low doses are adequately degraded or excreted from the body without adverse effects occurring. In the latter case, chronic studies may not be as critical in deriving an RfD as special studies assessing specific toxicity endpoints such as neurotoxicity or immunotoxicity (Cicmanec et al. 1996).

When uncertainties exist in one, two, and three of the areas just discussed, it is standard EPA practice to use a total composite UF of 10, 100, and 1,000, respectively (Cicmanec et al. 1996). When uncertainties exist in four areas it is

standard EPA practice to use a total UF of 3,000 "in recognition of the lack of independence of these factors" (USEPA 1994a). When five levels of uncertainty exist in the data, EPA recommends using a total uncertainty factor of not more than 10,000 in the derivation of an oral RFD (Cicmanec et al. 1996); however, in such cases an inhalation reference concentration is not derived (USEPA 1994a).

As noted, individual UFs of less than 10 can be used to derive an RFD. A value of 3 is routinely used for a UF intermediate between 1 and 10 because 3 is the approximate logarithmic mean of 1 and 10 and the assumption is made that the range of the UF is distributed log normally (USEPA 1994a; Cicmanec et al. 1996). If multiple UFs of 3 are used, the resulting total composite UF should not represent a level of precision greater than that inherent in the methodology (i.e., 3×3 is considered to be equal to 10; $3 \times 3 \times 3$ equals 30, and $3 \times 3 \times 3 \times 3$ equals 100).

A modifying factor (MF) may also be applied to the calculation of an RfD to account for uncertainties that are not specifically addressed by the individual UFs. For example, deficiencies in the key study, such as a statistically minimal sample size or poor exposure characterization, may warrant the use of an MF greater than 1. Conversely, allowance is made for the use of an MF of less than 1 if the use of the standard default UFs results in a RfD that, in the professional judgment of the risk assessors, is excessively low; this might occur in the case of essential elements. If all the uncertainties in the derivation of a RfD are addressed by the use of the five Uncertainty Factors, then the default value for the MF is 1.

V. Agent HD (Sulfur Mustard)

A. Toxicology

1. Acute Toxicity. Acute exposures to sulfur mustard can result in skin and eye damage, gastrointestinal irritation, and depressed myelopoiesis (resulting in leukopenia and anemia) (Vogt et al. 1984). Damage to the respiratory tract, which is the principal cause of mortality in the first few days to weeks after exposure to sulfur mustard, involves acute edema, inflammation, and destruction of the airway epithelial lining (Institute of Medicine 1993). Infection of the respiratory tract resulting in bronchopneumonia is a common complication of exposure to sulfur mustard.

The skin and eyes are especially sensitive to the toxic effects of sulfur mustard. When applied to human skin, about 80% of the dose evaporates and 20% is absorbed (Vogt et al. 1984). Skin penetration is at a rate of about 1–4 $\mu g\ cm^{-2}\ min^{-1}$ at a temperature of 75 °F (Renshaw 1946). About 12% of the amount absorbed remains at the site and the remainder is distributed systemically (Renshaw 1946). Doses to 50 $\mu g/cm^2$ cause erythema, edema, and sometimes small vesicles. Doses of 50–150 $\mu g/cm^2$ cause bullous-type vesicles, and larger doses cause necrosis and ulceration with peripheral vesication. Droplets of liquid sul-

fur mustard containing as little as 0.0025 mg may cause erythema (Ward et al. 1966). Eczematous sensitization reactions were reported in several early studies and may occur at concentrations less than those causing direct primary irritation (Rosenblatt et al. 1975). In humans, the LCt_{50} (estimated concentration × exposure time period lethal to 50% of exposed individuals) for skin exposures is 10,000 mg-min/m^3 (DA 1974) (this figure is for masked personnel; however, the amount of body surface area exposed was not reported). The ICt_{50} (estimated concentration × exposure time period incapacitating to 50% of exposed individuals) for skin exposures is 2000 mg-min/m^3 at 70 °–80 °F in a humid environment and 1000 mg-min/m^3 at 90 °F in a dry environment (DA 1974, 1992a). The ICt_{50} for contact with the eyes is 200 mg-min/m^3 (DA 1974, 1992a). The LD_{Lo} for skin exposure is 64 mg/kg and the LD_{50} is estimated to be about 100 mg/kg (DA 1974, 1992a).

Repeated exposure to 1.4 mg-min/m^3 produced no eye irritation or injury to laboratory animals (Rosenblatt et al. 1975). In humans, a Ct ≤12 mg-min/m^3 is considered a no-effect dose for eye irritation at ambient temperatures (McNamara et al. 1975). At higher temperatures (≥32 °C), threshold and other biological effects occur at lower concentrations. Cts of 12–70 mg-min/m^3 cause mild reddening of the eyes (McNamara et al. 1975); Cts of 40–90 mg-min/m^3 can cause eye irritation and conjunctivitis after a latency period of 2–48 hr; and Cts of 90–100 mg-min/m^3 produce moderately severe burns, ulcers, opacity, and perforation after a latency period of 2–10 hr (Doull et al. 1980). There may be recurrent vascularization and ulceration many years after the initial exposure.

The LCt_{50} for inhalation exposures in humans has been estimated to be 1500 mg-min/m^3 (DA 1992a). In animals, median lethal Ct values for sulfur mustard range from 600 to 1900 mg-min/m^3 for 10-min exposures (Rosenblatt et al. 1975). An LC_{Lo} (lowest lethal concentration) of 189 mg/m^3 per 10 min has been reported for mice (Lewis and Sweet 1984), and a 5-min LC_{Lo} of 77 ppm has been reported for dogs (ITII 1975).

Information on the acute oral toxicity of sulfur mustard is quite limited. The oral LD_{50} for humans has been estimated to be 0.7 mg/kg (DA 1992a). The oral LD_{50} for rats is 17 mg/kg (DA 1974). Rats treated with 2.5 mg kg^{-1} d^{-1} for 14 d developed inflammation, petechial hemorrhage, and thickening and sloughing of the gastric mucosa (Hackett et al. 1987).

2. *Subchronic Toxicity.* In a subchronic study conducted by Sasser et al. (1989a, 1996a) Sprague-Dawley rats (12 of each sex/group) were dosed by gavage with 0, 0.003, 0.01, 0.03, 0.1, or 0.3 mg sulfur mustard (in sesame oil)/kg body weight, 5 d/wk, for 13 wk. No mustard-related mortality occurred at any dose level. Body weights were significantly decreased in animals of the high-dose group. Epithelial hyperplasia of the forestomach occurred in 5/12 males and 5/12 females of the high-dose group and in 1/12 males receiving 0.1 mg kg^{-1} d^{-1}, but not in any other treatment group. A small number of squamous papillomas of the forestomach was also observed in about 10% of the intermediate (8/94) and high-dose (10/94) groups. Forestomach lesions were not observed

in any of the control animals. No other treatment-related pathological lesions, clinical chemistry changes, or hematological abnormalities were reported.

3. Chronic Toxicity. The U.S. Department of the Army (DA 1992a) has stated that chronic exposure to sulfur mustard can cause sensitization and chronic lung impairment (cough, shortness of breath, chest pain); however, specific information on dose–response functions for these effects was not found in the available literature. Limited information on the chronic toxicity of sulfur mustard comes from studies of workers at chemical agent manufacturing and weapons plants. Morgenstern et al. (1947) reported that many workers in a munitions plant handling sulfur mustard developed chronic bronchitis, which in some cases developed into bronchiectasis. Wada et al. (1962a,b) reported that a large proportion of the Japanese workers exposed to mustard, as well as to lewisite and several other agents, at a manufacturing plant during World War II exhibited productive cough, irregular fever, chronic bronchitis, emphysematous changes, and pleural adhesions. It is likely that in this case the reported effects were caused by concentrations of chemical agents sufficiently high to cause acute toxic effects; exposure to sulfur mustard was estimated to reach as high as 50–70 mg/m^3 at times (Inada et al. 1978).

McNamara et al. (1975) exposed male and female rats (140), mice (140), rabbits (12), guinea pigs (30), and dogs (6 initially) to a sulfur mustard vapor concentration of 0.001 mg HD/m^3 for 24 hr/d, 5 d/wk, for varying exposure durations up to 1 yr. (Note: the experimental protocol was described as a continuous exposure, implying that it was for 7 d/wk; however, the report specifically mentions that the exposures were for only 5 d/wk.) The same number of animals of each species were exposed to 0.1 mg HD/m^3 for 6.5 hr followed by 0.0025 mg HD/m^3 for 17.5 hr/d, 5 d/wk, for as long as 1 yr. The latter exposure is equivalent to a 5 d/wk time-weighted average concentration of 0.029 mg/m^3. Unexposed controls consisted of 10 dogs, 7 rabbits, 20 guinea pigs, 100 rats, and 120 mice. Exposed animals were killed periodically during the study and were replaced with new animals. Also, 100 mice were added to the test chambers about 6 mon after the tests began, and 50 A/J mice were added to the chambers about 3 mon later.

Signs of toxicity that could be attributed to the sulfur mustard exposure occurred only in rats and dogs. Of 39 rats exposed to 0.001 mg HD/m^3 for 12 mon, 5 exhibited chronic keratitis, a condition that McNamara et al. (1975) reported could possibly have been agent related; however, this effect was not observed in any rats exposed to 0.1 mg HD/m^3. No signs of toxicity were seen in any of the dogs exposed to 0.001 mg HD/m^3; however, it should be noted that only 2 animals were exposed for the full 52-wk period and only 4 animals were exposed for 32 wk. The major signs of toxicity seen in the dogs exposed to 0.1 mg HD/m^3 were ocular changes consisting of corneal opacity, pannus, vascularization, pigmentation, keratitis, and granulation. McNamara et al. (1975, p 12) stated that chronic keratitis and conjunctivitis occurred in 3 of 10 dogs

exposed for 7.5 or 12 mon. The tabulated data presented by McNamara et al. (Tables 8 and 9) indicate that chronic keratitis was also seen in some animals as early as 16 wk after exposure began, and may have occurred in as many as 5 of 10 animals exposed for 32, 40, or 52 wk. McNamara et al. (1975) concluded that it was "possible" that these effects were agent related. Pneumonitis occurred in several of the dogs exposed to 0.1 mg HD/m^3, but this condition was also seen in the control animals, and because no other respiratory tract lesions were found, McNamara et al. (1975) indicated that the observed pneumonitis was not agent related. There were no changes in blood chemistry of the exposed dogs except for a possible increase in serum glutamic oxaloacetic transaminase after 12–28 wk of exposure to 0.1 mg/m^3. As shown in Table 9, two dogs exposed to 0.1 mg HD/m^3 for 12 mon also exhibited anaphylactic syndrome, gastroenteritis, and petechia.

Table 8. Effects of sulfur mustard vapors on the eyes of dogs.[a]

Concentration (mg/m^3)	Exposure period (wk)	No. of dogs affected	Effects
0.001	4	0/10	NE
0.001	8	0/8	NE
0.001	16	0/6	NE
0.001	32	0/4	NE
0.001	52	0/2	NE
0.1 (1st group)	4	0/6	NE
0.1	8	0/4	NE
0.1	16	0/2	NE
0.1	28	2/2	Vascularization and pigmentation
0.1	40	1/2	Corneal opacity, pannus, chronic keratitis, granulation
0.1	40	1/2	Vascularization and pigmentation
0.1	52	2/2	Corneal opacity, pannus, chronic keratitis, granulation
0.1 (2nd group)	4	0/4	NE
0.1	8	0/4	NE
0.1	16	2/4	Corneal opacity, pannus, chronic keratitis, granulation
0.1	16	2/4	Vascularization and pigmentation
0.1	32	2/2	Corneal opacity, pannus, chronic keratitis, granulation
0.1	52	2/2	Corneal opacity

NE, no adverse effects.

[a]Exposures were for 5 d/wk.

Source: McNamara et al. (1975), Table A-18, p. 34.

Table 9. Toxicity of sulfur mustard vapors[a] to dogs.

No. of animals	Exposure (mon)	Post exposure (wk)	Gross findings	Microscopic findings
4	2	5	See microscopic findings	Splenic infarct (1/4); pneumonia, granulomatous, (1/4); pneumonitis, chronic (1/4)
1	4	4	NSL[b]	NSL
1	4 (control)	4	NSL	NSL
1	4	4	NSL	NSL
1	7.5	4	NSL	Keratitis, pigmentary; pneumonitis, chronic
1	7.5	4	NSL	Keratitis, chronic
1	7.5 (control)	4	NSL	Pneumonitis, chronic
1	7.5 (control)	4	NSL	Pneumonitis, chronic, active
1	12	10	Gastroenteritis; multiple petechiae; anaphylactic syndrome	Congestion, liver, spleen, lung; hemorrhage, pancreas; ulcerative colitis; keratitis, chronic; conjunctivitis, lymphocytic
1	12	10	Anaphylactic syndrome	Gastroenteritis, hemorrhagic; heart, petechia; keratitis, acute

[a]0.1 mg HD/m^3 for 6.5 hr followed by 0.0025 mg/m^3 for 17.5 hr/d, 5 d/wk.
[b]NSL, no significant lesions.
Source: McNamara et al. (1975), Table A-37, p. 53.

Although these effects were considered by McNamara et al. (1975) to be unrelated to the exposure to sulfur mustard, they are consistent with the known vesicant and sensitization actions of the agent. It is possible that the HD condensed on the fur of the animals and was subsequently ingested as a result of grooming behavior. Gastroenteritis could then have resulted from direct contact of the vesicant with the gastrointestinal epithelium.

4. Delayed Toxicity. Acute exposures to sulfur mustard can also result in long-term respiratory damage manifested as asthma-like conditions, emphysematous

bronchitis, and increases in incidence of secondary respiratory infections (bronchopneumonia and tuberculosis) (see review by Watson and Griffin 1992). Beebe (1960) evaluated the occurrence of respiratory tract disease among a group of World War I soldiers. Soldiers who had been exposed to mustard gas exhibited greater mortality from tuberculosis and pneumonia than either of two reference groups. Manning et al. (1981) reported a significantly increased incidence of mortality from pneumonia among 428 former workers of a sulfur mustard manufacturing facility; the ratio of observed to expected cases was 2 ($p < .05$). Some individuals exposed to sulfur mustard concentrations that are damaging to the eyes are susceptible to relapsing keratitis (delayed keratopathy) (see review by Watson and Griffin 1992). The condition may reappear 8–40 yr after recovery from the initial exposure (Dahl et al. 1985).

5. Developmental and Reproductive Effects. Azizi et al. (1995) investigated changes in serum concentrations of reproductive hormones and sperm counts in men who had been exposed to sulfur mustard during wartime. In 16 individuals, serum free and total testosterone and dehydroepiandrosterone were markedly decreased in the first 5 wk after exposure; but levels returned to normal by 12 wk. In 28 of 42 men evaluated 1–3 yr after exposure, sperm counts were less than 30 million cells/mL, and follicle-stimulating hormone was increased compared to controls having sperm counts greater than 60 million cells/mL. Testicular biopsy of the test subjects revealed partial or complete arrest of spermatogenesis. No information was provided on the possible effects of the exposure to sulfur mustard on reproductive success.

In a study conducted by Hackett et al. (1987), sulfur mustard (dissolved in sesame oil) was administered by intragastric intubation to rats and rabbits on gestation d 6–15 (rats) or 6–19 (rabbits). Female rats were dosed with 0, 0.2, 0.4, 0.8, 1.6, 2.0, or 2.5 mg kg^{-1} d^{-1} in a range-finding study (3–9 animals per dose group of which 2–7 per dose group were pregnant) and with 0, 0.5, 1.0, or 2.0 mg kg^{-1} d^{-1} in a teratology study (25–27 animals per dose group of which 20–26 per dose group were pregnant). Maternal and fetal toxicity was observed at all dose levels (Table 10). In the range-finding study significant ($p < .05$) maternal effects included mortality (1/3) at the highest dose; severe gastric lesions (petechial hemorrhage and sloughing of gastric mucosa) at 2.0 and 2.5 mg kg^{-1} d^{-1}; and inflamed mesenteric lymph nodes at doses of 0.4 mg kg^{-1} d^{-1} and higher. Significant decreases in body weight and decreased extragestational weight occurred at 1.6 mg kg^{-1} d^{-1} and decreased hematocrit at 0.8 mg kg^{-1} d^{-1}. There were no adverse effects on fetal weight and no evidence of morphological abnormalities in the fetuses. In the rat teratology study, maternal toxicity was evidenced by gastric inflammation at 2.0 mg kg^{-1} d^{-1}, and inflamed mesenteric lymph nodes at doses of 0.5 mg kg^{-1} d^{-1} and higher. Decreased body weight and decreased extragestational weight occurred at 0.5 mg kg^{-1} d^{-1}; decreased hematocrit at 1.0 mg kg^{-1} d^{-1}; and decreased weight of the placenta and gravid uteri at 2.0 mg kg^{-1} d^{-1}. Fetal effects included decreased weight in females and hydro-

Table 10. Lowest doses[a] of sulfur mustard causing maternal and fetal effects in rats and rabbits.

	Rat studies		Rabbit studies	
Effects	Range-finding (mg/kg)	Teratology (mg/kg)	Range-finding (mg/kg)	Teratology (mg/kg)
Maternal effects:				
Mortality	2.5	—	1.0	0.8
Gross lesions:				
Major[b]	2.0	—	1.0	0.4
Minor[c]	0.4	0.5	0.5	0.4
Decreased weight:				
Body	1.6	0.5	2.0	0.8
Extragestational	1.6	0.5	—	—
Extragestational gain	0.4	0.5	—	0.8[d]
Gravid uterus	—	2.0	—	—
Decreased hematocrit	0.8	1.0	—	0.8
Resorptions	0.4[e]	—	—	—
Fetal effects:				
Decreased weights:				
Female fetuses	—	0.5	2.0	—
Male fetuses	—	1.0	2.0	—
Placenta	—	2.0	—	—
Fetal morphology				
Misaligned sternebrae	—	2.0[f]	—	—
Supernumerary ribs	—	2.0[f]	—	—
Reduced ossification				
Vertebrae	—	0.5[e,g]	—	—
Sternebrae	—	2.0[f]	—	—
Hydroureter	—	0.5[e,f]	—	—

[a]Agent administered by intragastric intubation.
[b]Gastric lesions or infections.
[c]Inflamed mesenteric lymph nodes in rats; enlarged Peyer's patch in rabbits.
[d]Significantly different from lowest dose group, but not from controls.
[e]Not significant in the highest dose group.
[f]Significance based on fetal unit.
[g]Significance based on litter unit.
Source: Hackett et al. (1987).

ureter at 0.5 mg kg^{-1} d^{-1}; decreased weight of males at 1.0 mg kg^{-1} d^{-1}; and increased incidences of supernumerary ribs, misaligned sternebrae, and reduced ossification of sternebrae at 2.0 mg kg^{-1} d^{-1}. The investigators reported that the study did not reveal any evidence for a sulfur mustard-induced teratogenic effect in rats because all the observed fetal changes occurred at dose levels that also

produced maternal toxicity. The NOAEL for maternal and fetal toxicity was reported to be <0.5 mg kg^{-1} d^{-1}.

In the second part of the study by Hackett et al. (1987), rabbits were dosed with 0, 0.5, 1.0, 2.0, or 2.5 mg kg^{-1} d^{-1} in a range-finding study (7–8 per dose group), and with 0, 0.4, 0.6, or 0.8 mg kg^{-1} d^{-1} in the teratology study (7–8 per dose group). Dose levels of 0.8 mg kg^{-1} d^{-1} or higher were lethal to the dams. Damage to the gastric mucosa and enlarged Peyer's patches were observed in animals that received the lowest dose (0.4 mg kg^{-1} d^{-1}). Depressed body weight, depressed extragestational weight gain, and depressed hematocrit values occurred at 0.8 mg kg^{-1} d^{-1}. In the range-finding study a significant depression in fetal body weights occurred at a dose level of 2.0 mg kg^{-1} d^{-1}; however, in the teratology study no significant effects were observed on intrauterine survival, placental and fetal body weights, or incidence of fetal abnormalities. The investigators concluded that the study provided no evidence that sulfur mustard induced a teratogenic effect in rabbits. The NOAELs for maternal and fetal toxicity were reported to be <0.4 mg kg^{-1} d^{-1} and >0.8 mg kg^{-1} d^{-1}, respectively.

In a two-generation reproductive toxicity study conducted by Sasser et al. (1989b), groups of Sprague-Dawley rats (27 females and 20 males/group per generation) were gavaged with 0, 0.03, 0.1, or 0.4 mg kg^{-1} d^{-1}. The animals were treated according to the following exposure protocol: male and female rats were dosed five times per week for 13 wk before mating and during a 2-wk mating period; female rats were dosed daily throughout the 21-d gestation and parturition period; and females were dosed 4–5 times/wk during the 21-d lactation period. Males who had mated with females were killed at the birth of their pups; dams who had given birth were killed when the pups were weaned. Male and female F_1 pups received sulfur mustard until they were mated and the females became pregnant and gave birth. At this point, F_1 males were killed and F_1 dams continued on the dosage schedule until weaning, at which point the study was terminated. Thus, two generations of rats received subchronic exposure to sulfur mustard, with each generation going through a mating cycle. Similarly, two generations of pups were born to parents who had received sulfur mustard.

Body weight gain was significantly ($p < .05$) lower than control values in the F_1 rats of both sexes born to parents who had received the highest dose of sulfur mustard. There were no significant adverse effects on reproductive parameters at any dose level. However, dose-related lesions of the squamous epithelium of the forestomach (acanthosis and hyperplasia) occurred in both sexes of each treatment group. The lesions were described as mild at the lowest dose level, 0.03 mg/kg, compared with the higher-dose groups. The incidence and severity of acanthosis was 0/94 in the controls, 71/94 in the low-dose group, 89/94 in the mid-dose group, and 94/94 in the high-dose group. Benign neoplasms of the forestomach occurred in 8/94 animals in the 0.1 mg/kg group and in 10/94 animals of the 0.4 mg/kg group. The results of this study indicate that the lowest dose tested (0.03 mg kg^{-1} d^{-1}) is a LOAEL for maternal toxicity.

McNamara et al. (1975) reported no increased fetal mortality rate when groups of 10 rat dams were exposed by inhalation to 0.001 mg HD/m^3, 24 hr/d,

or to 0.1 mg HD/m^3 for 6.5 hr followed by 0.0025 mg HD/m^3 for 17.5 hr during the first, second, or third week, or the entire period of gestation. In another study, groups of 10 unexposed female rats were bred to male rats that had been exposed to the same exposure concentrations of HD for 1, 2, 4, 8, 24, 36, or 52 wk to gain information on dominant lethal mutagenesis. There was no evidence of mutagenesis, and fetal mortality was considered within normal limits. Both studies had a number of shortcomings; in particular, the authors stated that the fetuses were examined, but they did not indicate whether there were fetal abnormalities.

6. Carcinogenicity. Several studies on workers occupationally exposed to sulfur mustard have revealed elevated risks of respiratory tract and skin tumors after long-term exposure. In addition, animal studies, mutagenicity studies, genotoxicity data, and the fact that sulfur mustard is a potent DNA alkylating agent all provide supporting evidence for the carcinogenicity of this chemical agent.

The International Agency for Research on Cancer (IARC) has classified "mustard gas" as a Group 1 carcinogen (IARC 1987a), and the National Toxicological Program (NTP) includes "mustard gas" in the category of "Substances or groups of substances, occupational exposures associated with a technological process, and medical treatments that are known to be carcinogenic" (NTP 1994; *Annual Report on Carcinogens*). The state of Maryland also considers "mustard gas" as a "known human carcinogen" (a Class I.A. Toxic Air Pollutant as defined by the Code of Maryland Regulations, CMR Title 26 Subtitle 11, as amended).

Human Data. IARC (1975), Waters et al. (1983), Watson et al. (1989a), and the Institute of Medicine (1993) have summarized the epidemiological evidence concerning the potential carcinogenicity of sulfur mustard in humans. Much of this information has come from studies of soldiers exposed during World War I as well as from studies of workers at chemical agent manufacturing facilities.

Case and Lea (1955) reported 29 deaths from cancer of the lungs and pleura among a sample of 1267 World War I veterans who had been exposed to sulfur mustard, 80% of whom also suffered from chronic bronchitis. In comparison, 14 cases would have been expected in a population of that size based on the mortality rates for the male population of England and Wales. The mortality ratio (ratio of "deaths found" to "deaths expected") for respiratory tract cancers was 2.07 (p between .0001 and .01). A similar tumor incidence rate and mortality ratio (2.01) were found in a population of veterans who had never been exposed to mustard but who were suffering from bronchitis. Case and Lea (1955) concluded that the evidence did not support the view that sulfur mustard was a direct carcinogen. IARC (1975), however, noted that the high tumor rate in the group not exposed to mustard may have been caused, in part, by smoking habits (a significantly higher proportion of men injured by mustard gas had given up smoking by age 40).

Beebe (1960) evaluated the occurrence of respiratory tract cancers among a group of 2718 American soldiers exposed to sulfur mustard during World War I and found that the ratio of observed to expected cases was 1.47 (based on U.S. mortality rates), compared with 1.15 for wounded soldiers not exposed to sulfur mustard and 0.81 for soldiers who had pneumonia but who had not been exposed to mustard. Norman (1975) evaluated the same group of soldiers after a 10-yr follow-up period (study completed in 1965) and found that the exposed men had a 40% excess of lung cancer mortality, with an estimated relative risk of 1.3 (95% confidence limits, 0.9–1.9) compared to a control group consisting of wounded soldiers without exposure to mustard. The latency period was estimated to be 22–37 yr. Norman (1975) also reported that in a limited subgroup of veterans, the relative risk of lung cancer mortality among cigarette smokers who were exposed to mustard agents was approximately equal to that of veterans exposed to mustard who stated that they did not smoke (4.3 vs. 4.4). Norman (1975) concluded that there was no evidence in this limited data set that mustard exposure and cigarette smoking had a synergistic effect on lung cancer mortality.

Retrospective studies of Japanese workers who had been employed at a chemical agent manufacturing plant from 1929 to 1945 have revealed that these individuals have an increased risk of developing respiratory tract cancers. Although sulfur mustard was the main product of the facility, lewisite, diphenylarsine, hydrocyanic acid, phosgene, and chloracetophenone were also produced there (Inada et al. 1978), and it is not known to what degree these other chemicals contributed to the observed effects. The concentration of mustard in the workplace was estimated to be as high as 50–70 mg/m^3 (Nakamura 1956), and reportedly the workers frequently exhibited signs of mustard toxicity including acute conjunctivitis, acute rhinitis, acute bronchitis, and acute dermatitis with blister formation. Studies completed in the 1950s documented individual cases of bronchial and laryngeal carcinoma in this population of workers (Yamada et al. 1953, 1957). Yamada (1963) reported that 16.3% of 172 deaths of former workers were caused by cancers of the respiratory tract and oropharynx. In contrast, the incidence rate among 5030 nonexposed inhabitants from the same geographic area was reported to be 0.4% (Yamada 1963). Mortality rates among the former factory workers during the years 1952–1967 were studied by Wada et al. (1968) who found that the incidence of mortality from respiratory tract cancer was 33/495 (30 confirmed by histological evaluation), compared to an expected 0.9 on the basis of national mortality rates for males with the same age distribution as the mustard workers. Of 930 former factory workers not directly involved in the mustard production process, 3 had died of respiratory tract cancer compared to 1.8 expected. Neoplasms occurred in the tongue, pharynx, sphenoidal sinus, larynx, trachea, and bronchi; only one occurred peripherally in the lung. The median length of employment was 7.4 yr, and the median interval between first employment and death from cancer of the respiratory tract was 24.4 yr (Wada et al. 1968).

Additional studies of this population of workers were conducted by Nishi-

moto et al. (1983, 1988), who incorporated histopathological and mortality data gathered between 1952 and 1986. For 1632 of these workers, the standardized mortality ratio (SMR) for respiratory tract tumors was 3.9 [70 observed vs. 17.8 expected ($p < .001$) based on data for the Japanese male population) and the SMR for all malignant tumors was 1.2 (173 observed vs. 142 expected, $p < .01$)]. These individuals were divided into three groups: (A) those directly involved in the manufacture of sulfur mustard or lewisite; (B) those not involved in mustard or lewisite manufacture, but who experienced some exposure; and (C) those engaged in the manufacture of other gases and those who were never exposed. The SMR for groups A and B (1.6 and 1.9) were also significantly elevated ($p < .001$) whereas that for group C was not. Nishimoto et al. (1988) also showed that the SMR was about 2.7 for individuals who had worked at the factory for 0.5–5 yr, but 7.17 for individuals who had been employed for more than 5 yr. The SMR was not significantly elevated for individuals who had worked at the factory for 7 mon or less. SMRs were also calculated for each of six age groups. For individuals 30–39 yr old, the SMRs for respiratory tract cancer were not significantly elevated; however, the SMRs for the groups 40–49, 50–59, 60–69, and 70–79 yr old were 10.3, 3.9, 4.4, and 2.5, respectively (all were statistically significant at $p < .01$ or $p < .001$).

In a continuation of these studies, Yamakido et al. (1996) incorporated cancer incidence data up to 1992. Multivariate regression analysis was used to eliminate the effects of confounding factors, such as tobacco smoking habits, age, and duration of employment. As in the previous studies, the death rates from total malignancies ($p < .01$) and lung cancer ($p < .001$) were significantly higher in the exposed group; the SMR was slightly greater than 1 for all malignancies and almost 4 for lung cancer. For those individuals who had worked at the factory more than 5 years, the SMRs for lung cancers were 7.35, 4.92, and 1.5, and the SMRs for total malignancies were 2.36, 1.66, and 0.75 for groups A, B, and C, respectively.

Histopathological studies conducted by Yamada (1974, as reported by Inada et al. 1978) on 94 autopsy cases and 8 surgical cases revealed 17 cases of digestive tract cancers among these workers (no comparisons with control groups were reported). Yamakido et al. (1996) later reported 85 cases of malignant digestive tract neoplasms in group A workers, 62 in group B, and 37 in group C; however, the death rate from such cancers was not significantly elevated in the exposed workers (SMR <1).

Of the 488 former workers examined dermatologically, 115 had abnormal pigmentation and 22 had skin tumors of which 8 were cases of Bowen's disease (Inada et al. 1978). Pigmentation disorders were present in 57 cases of 109 engaged only in the production of mustard and in only 1 of 16 cases engaged only in the production of lewisite. Hyperkeratotic skin lesions such as Bowen's disease, basal cell carcinomas, and hyperkeratotic papular eruptions were present in 14 cases of 109 engaged only in mustard production and in 1 case of 16 engaged only in lewisite production. No abnormalities were observed in 77 for-

mer factory workers who had no exposure to chemical agents (Inada et al. 1978). It was also observed that the longer an individual had been exposed to mustard, the more marked the skin lesions tended to become (Inada et al. 1978).

Weiss and Weiss (1975) conducted studies evaluating the health of 271 workers employed for varying lengths of time between 1935 and 1945 at a munitions depot where the production, testing, and destruction of sulfur and nitrogen mustard (as well as bromoacetone, phosgene, chloropicrin, and organic arsenicals) had occurred. Of the group, 90% had chronic health problems and 114 had died by the end of 1974; 35% died of cancer, of which 38% were bronchial cancers. The total number of deaths from cancer was significant ($p < .01$), and the number of bronchial cancers was also significant, 11 observed vs. 5 expected for the population of the geographic region where the facility was located. The number of cancers of the gastrointestinal tract was 35% greater than expected. The average tumor induction time was 21.6 yr. IARC (1975) noted that the study was limited to workers with available medical records, which "raises the possibility that the proportion with cancer may have been inflated, since medical records or autopsy records would more likely have been preserved for workers with cancer." Furthermore, IARC (1975) does not indicate whether smoking habits and other confounding factors were accounted for in the study of Weiss and Weiss (1975).

According to Klehr (1984), German workers involved in the dismantling of a sulfur mustard facility developed multiple skin lesions including basal cell carcinomas, Bowen's disease, and carcinoma spinocellulare (a histological characterization of an epidermal carcinoma). The incidence rate for all tumors, including skin tumors, was 34% in 53 workers evaluated.

Manning et al. (1981) evaluated the incidence of cancer among former workers of a British mustard manufacturing facility (1939–1945). As of 1974, the number of deaths from all neoplasms combined (45) was slightly greater than that expected from national death rates, but the increase was not statistically significant. Two deaths were attributed to cancer of the larynx and 1 to carcinoma of the trachea, compared with an expected number of 0.40 ($p < .02$; relative risk, 7.5). Seven individuals were known to have developed cancer of the larynx, compared with 0.75 expected ($p < .001$; relative risk, 9.3). Lung cancer deaths were also elevated (21 observed vs. 13.43 expected) but not to significant levels (relative risk, 1.6). In follow-up investigations of this group of workers, Easton et al. (1988) evaluated the mortality records of 3354 individuals and found greater numbers of cancer deaths when compared to national mortality rates. Significant increases were observed in deaths from cancer of the larynx (11 observed, 4.04 expected; $p = .003$), pharynx (15 observed, 2.73 expected; $p < .001$), and all other buccal cavity and upper respiratory sites combined (12 observed, 4.29 expected; $p = .002$). There were also 200 deaths from lung cancer compared with 138.39 expected ($p < .001$). It was also reported that the risks of developing cancer of the lung and pharynx were significantly related to the duration of employment. Significant excess mortality was also observed for can-

cers of the esophagus (20 observed vs. 10.72 expected) and stomach (70 observed vs. 49.57 expected), but there was no correlation with time since first exposure or duration of exposure.

Manning et al. (1981) concluded that it was very likely that the observed cancers of the pharynx, larynx, and other upper respiratory sites resulted from exposure to sulfur mustard because the excesses were too large to be accounted for by confounding factors (the effects of smoking, however, were not evaluated), increased with increasing duration of employment, and were limited to the period more than 10 years after first employment. Evidence for a causal relationship between sulfur mustard exposure and other cancers, including lung cancer, was not considered to be as strong.

Although a large number of American military personnel were exposed to sulfur mustard in chamber and field tests conducted during World War II, the morbidity and mortality records of this cohort have not been adequately evaluated to document long-term health risks (Institute of Medicine 1993).

Animal Studies. McNamara et al. (1975) exposed Sprague-Dawley–Wistar rats, ICR Swiss albino and A/J mice, rabbits, guinea pigs, and dogs to sulfur mustard vapors for varying exposure durations up to 1 yr. The test animals were exposed to 0.001 mg HD/m^3 continuously or to 0.1 mg HD/m^3 for 6.5 hr, followed by 0.0025 mg HD/m^3 for 17.5 hr/d, 5 d/wk. In the rat study, 70 males and 70 females were exposed at each of the two concentrations, and 50 of each sex were maintained as controls. No tumors were observed in rabbits, guinea pigs, dogs, or mice; however, skin tumors were seen in the rats and these were considered to result from exposure to sulfur mustard. The rats were tested in two separate studies: a "toxicity study" in which the animals were exposed for as long as 52 wk and then followed for 6 mon, at which time they were killed, and a "carcinogenicity study" in which the animals were exposed for varying times and then observed for varying periods of time before being killed. In both studies skin tumors occurred in animals exposed to the highest concentration, but not in those exposed to the lower concentration. Of the tumors observed in the exposed animals, McNamara et al. (1975) considered basal cell and squamous cell carcinomas, trichoepitheliomas, and keratoacanthomas of the skin to be related to sulfur mustard exposure; the incidence of these tumors is shown in Tables 11 and 12.

Heston (1950) reported an increase in the occurrence of pulmonary tumors in strain A mice injected intravenously with 0.25 mL of a 1 : 10 dilution of a saturated solution of HD in water (0.06%–0.07%) at 2-d intervals for a total of 4 doses. The tumor incidence was 93.3% with 2.6 tumors/mouse compared with 61% in the controls (0.9 tumors/mouse). In a second test in which a slightly lower dose was used, pulmonary tumors were found in 68% of the surviving treated animals (1.09 tumors/mouse) compared with 13% in the controls (0.13 tumors/mouse) ($p < .001$). A significant increase in the incidence of pulmonary tumors in strain A mice was also seen in an inhalation study in which the test animals were exposed for 15 min to vapors released from 0.01 mL of HD ap-

Table 11. Incidences of skin tumors in the McNamara et al. (1975) toxicity study.

Exposure duration (mon)	Post exposure (d)	Control		0.001 mg/m^3		0.1/0.0025 mg/m^3	
		M	F	M	F	M	F
2			0/5	0/5	0/5	0/5	
3		0/5	0/5	0/5	0/5	0/5	0/5
4					0/5	0/5	
6		0/5	0/5	0/5	0/5		
8		0/5	0/5	0/5	0/5	0/5	0/5
12		0/5	0/5	0/5	0/5[a]	0/5	0/5
12	70					4/4[4b]	
12	90	0/4	0/4[c]	0/4	0/5	0/1	0/5
12	180	0/7	0/4[a,d]	0/6	0/14[c]	0/6	5/13[4b,f,g]

Note: Superscripts indicate the number and types of tumors: a, subcutaneous fibroma; b, skin, squamous cell carcinoma; c, squamous cell carcinoma of uterus; d, pulmonary adenoma; e, papilloma of the skin; f, basal cell carcinoma of the skin; g, thyroid adenoma. Only tumor types, b and f, were considered by the authors to be related to the HD exposure, and only these types are counted in the numerators.

Source: McNamara et al. (1975); adapted by USEPA (1991a).

plied to filter paper [Heston and Levillain (1953); exposure levels were not otherwise quantified]. Eleven months after exposure, lung tumor incidence was 49% (33/67) in the exposed animals and 27% (21/77) in the controls ($p < .01$).

In another study, Heston (1953) found that subcutaneous injections of HD (0.05 mL of a 0.05% solution at weekly intervals for 6 wk, or 0.1 mL of a 0.1% solution in olive oil at 2-d intervals for a total of 6 doses) into the middorsal region of mice (strains A, C3H, and C3Hf) resulted in injection-site tumors, whereas injections of vehicle alone did not induce tumor formation. Tumors occurring at the injection site included sarcomas, sarcomas neurogenic in origin, a rhabdomyosarcoma, papillomas, a squamous cell carcinoma, a hemangioendothelioma, and a mammary carcinoma.

7. Genotoxicity. IARC (1975), Fox and Scott (1980), and ATSDR (1992) have summarized the available evidence concerning the genotoxicity of sulfur mustard. Because sulfur mustard is a strong DNA alkylating agent, genotoxic effects occur through cross-link formation, inhibition of DNA synthesis and repair, point mutations, and chromosome and chromatid aberrations (ATSDR 1992). Some of these conditions have been observed in humans following exposure to sulfur mustard, others in various test systems including bacteria, yeast, insects, and mammalian cell cultures.

In studies conducted on a group of 28 former employees of a chemical agent manufacturing plant, Yanagida et al. (1988) found that the frequency of mutations to hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) deficiency was significantly elevated when compared to two control groups matched for

Table 12. Incidences of skin tumors in McNamara et al. (1975) cancer study (data for both sexes pooled).[1,2]

Exposure duration (wk)	Post exposure (mon)	Control	0.001 mg/m^3	0.1 mg/m^3
1	13		0/1	
1	15			0/1[a]
1	21		0/4[b]	0/4
2	20		0/5	0/5[c]
4	16		0/1	0/1
4	20		0/4	0/5
8	15	0/4	0/2	0/4
8	17		0/1	
8	18		0/1[d]	
12	12		0/2	4/5[3f,g]
12	17		0/3[e]	
26	14		0/4	3/4[3f]
26	18		1/1[f]	
39[3]	11		0/3[e]	4/4[4f,h]
52	2			1/1[f]
52	4			1/1[h]
52	6			1/1[f]
52	7			0/1
52	10	0/22[e]	0/17	3/14[3e,2f,i]
52	17	0/1[e]		0/1[e]
52	18			4/4[f]

[1]Superscripts indicate the number and types of tumors: a, subcutaneous lipoma; b, axillary lipoma; c, subcutaneous fibroma; d, astrocytoma; e, skin, fibroma; f, skin, squamous cell carcinoma; g, skin, basal cell carcinoma; h, skin, trichoepithelioma; i, skin, keratoacanthoma.

[2]Only types f, g, h, and i, were considered by the authors to be related to the HD exposure and only these types are counted in the numerators.

[3]At 0.1 mg/m^3, one type h tumor cooccurred in one animal with a squamous cell carcinoma.

Source: McNamara et al. (1975); adapted by USEPA (1991a).

age and smoking status. One control group consisted of healthy men and the other of individuals with bronchitis. The data also showed that the mutations were significantly more frequent in those workers who had longer exposures to sulfur mustard. A chromosome study of 16 former workers of this same factory indicated a significantly higher incidence of sister chromatid exchanges (SCE) in peripheral lymphocytes when compared to a control group ($p < .03$) (Shakil et al. 1993). Two individuals with chronic myelocytic leukemia had an almost threefold higher SCE rate than controls and also a high (12.1%) incidence of chromosome abnormalities (Shakil et al. 1993). In an evaluation of the p53 mutations found in lung tumors of sulfur mustard workers, Takeshima et al.

(1994) found that the mutations were similar to those in lung tumors of tobacco smokers (the factory workers were also tobacco smokers); however, the prominence of G:C to A:T transitions and the occurrence of double mutations in 2 of 12 cases suggested that the exposure to sulfur mustard did contribute to the development of the lung cancers.

Wulf et al. (1985) reported significant ($p < .001$) increases in sister chromatid exchanges in lymphocytes of 11 fisherman who had accidently been exposed to sulfur mustard in sufficiently high concentrations to cause signs of acute toxicity.

Sulfur mustard has been found to be genotoxic and mutagenic in several microbial assays. The agent caused alkylation of DNA in the yeast *Saccharomyces cerevisiae* (Kircher and Brendel 1983) and interstrand DNA cross-links (Venitt 1968) and inhibition of DNA synthesis (Lawley and Brookes 1965) in *Escherichia coli*. Using the histidine reversion assay, Stewart et al. (1989a) found that sulfur mustard induced point mutations in *Salmonella typhimurium* strain TA102 and frameshift mutations in TA 97 but neither type of mutation in strains TA98 and TA100.

Sulfur mustard inhibited DNA synthesis in mouse lymphoma cells (Crathorn and Roberts 1965), HeLa cells (Crathorn and Roberts 1966), and L-strain mouse fibroblasts (Walker and Thatcher 1968). It also induced chromosomal aberrations in cultured rat lymphosarcoma and mouse lymphoma cells (Scott et al. 1974) and chromosomal aberrations and reverse mutations in male BDF_1 mice in a host-mediated assay using murine leukemia L5178Y/Asn$^-$ cell line as an indicator (Capizzi et al. 1973).

Several studies have also demonstrated that sulfur mustard causes dominant lethal mutations. Rozmiarek et al. (1973) reported a dominant lethal mutation rate of 9.4% (±1.9%) in rats after adult males had been exposed to 0.1 mg HD/m^3 for 12 wk. Sasser et al. (1990) reported that a dominant lethal effect occurred after male Sprague-Dawley rats were dosed orally with 0.5 mg HD kg^{-1} d^{-1}, 5 d/wk, for 10 wk. The observed effects included increases in early fetal absorptions, preimplantation losses, and decreases in total live embryo implants. A significant increase in the percentage of abnormal sperm was also reported. Dominant lethal mutations, as well as chromosome rearrangements, have also been observed in *Drosophila melanogaster* exposed to sulfur mustard (Auerbach and Robson 1946).

The cytotoxic, clastogenic, and mutagenic effects of HD in Chinese hamster ovary cells have also been evaluated by Jostes et al. (1989a). Chromosomal aberration frequency increased in a dose-dependent manner over the dose range of 0.0625 to 0.25 μM. Mutation induction at the HGPRT locus was sporadic, but the majority of the exposures resulted in mutation frequencies that were 1.2- to 4.0 fold higher than the spontaneous frequencies.

B. Estimated Reference Dose

1. Selection of the Key Study. The available toxicity data for sulfur mustard are summarized in Table 13 The experimental study considered most suitable

Table 13. Toxicity data for sulfur mustard.

Study	Type	NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Comments
Hackett et al. (1987)	Rabbit teratology	>0.8	—	No fetal toxicity
Hackett et al. (1987)	Rabbit teratology	—	0.4	Maternal toxicity
Hackett et al. (1987)	Rat teratology	—	0.5	Maternal and fetal toxicity
Sasser et al. (1989a)	Rat subchronic	0.1	0.3	Epithelial hyperplasia of the forestomach
Sasser et al. (1989b)	Rat two-generation	—	0.03	Maternal toxicity; acanthosis and hyperplasia of the forestomach
Sasser et al. (1989b)	Rat two-generation	0.4	—	Reproductive effects

NOAEL, no-observed-adverse-effect level; LOAEL, lowest-observed-adverse-effect level.

for the derivation of an oral RfD_c for sulfur mustard is the rat two-generation reproductive toxicity study conducted by Sasser et al. (1989b). This study, extending over 42 wk, provides the lowest LOAEL. Acanthosis and hyperplasia of the epithelial tissue of the forestomach were the critical endpoints. Forestomach lesions have also been observed in other oral toxicity studies on sulfur mustard (Sasser et al. 1989a, 1996a). Some investigators have noted that the absence of a forestomach in humans suggests that the rat may not be a reliable human surrogate for evaluating the toxic effects of sulfur mustard. In addition, effects of gavage treatment in which the tissues are in immediate contact with sulfur mustard dissolved in sesame oil may not be comparable to the more dispersed contact expected when sulfur mustard is administered in drinking water or food.

2. *RfD_c Derivation.* In the study by Sasser et al. (1989b), male and female Sprague-Dawley rats were gavaged with 0, 0.03, 0.1, or 0.4 mg kg^{-1} d^{-1} dissolved in sesame oil. There was no evidence of adverse reproductive effects at the dose levels tested. There was a significantly ($p < .05$) decreased body weight gain in the F_1 rats of both sexes born to parents who had received the highest dose of sulfur mustard. In addition, dose-related lesions of the squamous epithelium of the forestomach (acanthosis and hyperplasia) occurred in both sexes of each treatment group. The lesions were described as mild at the lowest dose level, 0.03 mg kg^{-1} d^{-1}, compared with the higher-dose groups. The incidence and severity of acanthosis was 0/94 in the controls, 71/94 in the low-dose group, 89/94 in the mid-dose group, and 94/94 in the high-dose group. Benign neoplasms of the forestomach occurred in 8/94 animals in the 0.1 mg kg^{-1} d^{-1} group and in 10/94 animals of the 0.4 mg kg^{-1} d^{-1} group. The investigators reported that the NOAEL for toxicity was <0.03 mg/kg and the NOAEL for reproductive effects was >0.4 mg kg^{-1} d^{-1}.

The lowest dose tested, 0.03 mg kg^{-1} d^{-1}, can be considered a LOAEL for rats subchronically exposed to sulfur mustard, with epithelial acanthosis and hyperplasia of the forestomach as the critical effect. Using this LOAEL, a human chronic RfD_c can be derived by adjusting the dose to a 7 d/wk exposure protocol and then applying the result to the RfD methodology. Dose adjustments for discontinuous exposure can be made as follows: female rats were gavaged five times per week for 15 wk (75 d), total dose = 2.25 mg/kg; daily for 3 wk (21 d), total dose = 0.63 mg/kg; and four times per week for 3 wk (12 d), total dose = 0.36 mg/kg. The combined total dose over the 21-wk exposure period, therefore, was 3.24 mg/kg; dividing the combined total dose of 3.24 mg/kg by 147 d (21 wk) results in an adjusted LOAEL of 0.022 mg kg^{-1} d^{-1}. The adjusted LOAEL can then be applied to the equation for the derivation of an RfD:

$$\text{RfD} = \frac{\text{LOAEL}}{\text{UF}_\text{H} \times \text{UF}_\text{A} \times \text{UF}_\text{L} \times \text{UF}_\text{S} \times \text{UF}_\text{D} \times \text{MF}}$$

$$RfD_e = \frac{0.022 \text{ mg/kg/day}}{10 \times 10 \times 3 \times 10 \times 1 \times 1}$$

$$RfD_e = 0.077 \text{ µg HD/kg/day}$$

where:

LOAEL = 0.022 mg kg^{-1} d^{-1}
UF_H = 10 (to protect sensitive individuals)
UF_A = 10 (for animal to human extrapolation)
UF_L = 3 (for estimating the NOAEL from the LOAEL)
UF_S = 10 (for subchronic to chronic extrapolation)
UF_D = 1 (data base adequate)
MF = 1 (no additional modifications needed).

A uncertainty factor of 3000 was applied, accounting for protection of sensitive subpopulations (10), animal-to-human extrapolation (10), LOAEL-to-NOAEL extrapolation (3), and extrapolation from a subchronic to chronic exposure (10).

A LOAEL-to-NOAEL uncertainty factor of 10 is not considered to be necessary because the observed effect was reported to be "mild"; the critical effect may have been enhanced by the vehicle used (sesame oil in which sulfur mustard is fully soluble) and the route of administration (i.e., gavage is more likely to result in localized irritant effects). Although the target organ (rat forestomach) is of questionable relevance to humans, because sulfur mustard is a vesicant and direct alkylating agent, tissue damage would be expected to occur at the point of contact regardless of location in the gastrointestinal tract.

The data base for sulfur mustard contains two developmental toxicity studies in different species, a reproductive bioassay and a standard subchronic toxicity study in one species. In addition, chronic inhalation studies have been conducted on sulfur mustard using rats, mice, guinea pigs, and dogs. The principal study identified a toxic effect that is consistent with the vesicant properties of sulfur mustard. There is no evidence that any other experimental species would be more sensitive to ingested sulfur mustard; therefore, additional oral toxicity studies in other species are not considered critical.

3. Confidence in the RfD_e.

Study: High
Data base: Medium
RfD: Medium

The principal study is a well-designed and well-conducted reproductive toxicity study in rats. The identified endpoint (gastric lesions), also identified in a subchronic study and in two developmental studies, supports the conclusion that direct contact with epithelial tissue is the primary mechanism of toxicity. How-

ever, the principal study did not identify a NOAEL for this effect, and the route of administration (gavage) may have led to an enhanced response because of the bolus type of dosing. Consequently, the overall confidence in the RfD_c must be considered medium.

VI. Agent HT (Sulfur Mustard)

Agent HT is a chemical mixture consisting of HD and agent T, plus some sulfur impurities. When agent HT was first produced, the ratio of HD to T was about 60 : 40; however, the proportion changes with time because of agent degradation and mixing with impurities.

A. Toxicology

1. Acute Toxicity. Because agent HT is a chemical mixture of HD and agent T, it is likely that HT has many of the same toxic effects as HD. However, very few toxicity studies specific for agent HT were found in the available literature.

Like agent HD, HT is a vesicant, causing blistering on the skin after a latency period of 4–24 hr (DA 1990b). Exposure of the eyes results in lacrimation, photophobia, inflammation of the conjunctiva and cornea, and necrosis. Inhalation of the vapor or aerosol can result in inflammation of the upper respiratory tract, sneezing, coughing, bronchitis, loss of appetite, diarrhea, fever, and apathy (DA 1990b). Exposure to nearly lethal doses can cause injury to the bone marrow, lymph nodes, and spleen with a resulting increased risk of local and systemic infections. Ingestion of HT can result in severe stomach pains, vomiting, and bloody stools. LCt_{50}s of 100–200 mg-min/m^3 (dog), 3000–6000 mg-min/m^3 (guinea pig and rabbit), and 820 mg-min/m^3 (mouse) have been reported (DA 1990b).

2. Subchronic Toxicity. No data were found in the available literature on the subchronic toxicity of agent HT in animals or humans.

3. Chronic Toxicity. According to the U.S. Department of the Army (DA 1990b), chronic exposure to HT can cause sensitization and chronic lung impairment (cough, shortness of breath, chest pain); however, epidemiological data or experimental animal data to evaluate dose–response functions for such effects were not found in the available literature.

4. Developmental and Reproductive Effects. According to the U.S. Department of the Army (DA 1990b), chronic exposure to HT can cause birth defects; however, specific teratogenicity and developmental toxicity studies on agent HT were not found in the available literature.

5. Carcinogenicity. HD is a known carcinogen and therefore HT, which contains 60% HD, may also have some carcinogenic potential. According to the

U.S. Department of the Army (DA 1990b), chronic exposure to HT can cause cancer of the mouth, throat, respiratory tract, and skin, as well as leukemia. No experimental animal carcinogenicity studies on HT were found in the available literature.

6. Genotoxicity. Because HT contains 60% HD, it is likely to have genotoxic effects similar to those for HD (sulfur mustard). In addition, the second component of HT, agent T, may also have genotoxic activity. A mutagenicity study conducted by Auerbach and Robson (1947a) indicated that the potency of agent T to produce sex-linked lethal mutations in *Drosophila melanogaster* (fruit fly) is comparable to that of HD.

B. Estimated Reference Dose

Insufficient toxicity data are available to derive an RfD_c for HT. Because HT contains 60% agent HD, its toxicity is likely very similar to that of agent HD. However, the uncertainties associated in assessing the potential contribution of agent T to the toxicity of HT prevent derivation of a verifiable RfD.

VII. Agent T

A. Toxicology

1. Acute Toxicity. Agent T [bis(2(2-chloroethylthio)ethyl) ether] is a component of agent HT and represents about 40% of the mixture. No studies assessing the short-term toxicity of agent T following oral exposure were found in the available literature. It is likely that agent T has toxic effects similar to those of HT. Agent HT is a vesicant, causing blistering on the skin after a latency period of 4–24 hr (DA 1990b). Exposure of the eyes results in lacrimation, photophobia, inflammation of the conjunctiva and cornea, and necrosis. Inhalation of the vapor or aerosol can result in inflammation of the upper respiratory tract, sneezing, coughing, bronchitis, loss of appetite, diarrhea, fever, and apathy (DA 1990b). Exposure to nearly lethal doses can cause injury to the bone marrow, lymph nodes, and spleen with a resulting increased risk of local and systemic infections. Ingestion of HT can result in severe stomach pains, vomiting, and bloody stools. LCt_{50}s of 100–200 mg-min/m^3 (dog), 3000–6000 mg-min/m^3 (guinea pig and rabbit), and 820 mg-min/m^3 (mouse) have been reported for agent HT (DA 1990b). Although agent T has been considered a relatively weak vesicant, the LCt_{50} (40 mg-min/m^3) for agent T indicates that it may contribute to the overall toxicity of agent HT (Robinson 1967; Watson and Griffin 1992).

2. Subchronic Toxicity. No data were found in the available literature on the subchronic toxicity of agent T in animals or humans.

3. Chronic Toxicity. According to the U.S. Department of the Army (DA 1990b), chronic exposure to HT can cause sensitization and chronic lung impair-

ment (cough, shortness of breath, chest pain); however, epidemiological data or experimental animal data to evaluate dose–response functions for such effects for agent T were not found in the available literature.

4. Developmental and Reproductive Effects. According to the U.S. Department of the Army (DA 1990b), chronic exposure to HT can cause birth defects; however, specific teratogenicity and developmental toxicity studies on agent T were not found in the available literature.

5. Carcinogenicity. Information regarding the carcinogenic potential of agent T in humans or animals was not found in the available literature.

6. Genotoxicity. A mutagenicity study conducted by Auerbach and Robson (1947a) indicated that the potency of agent T to produce sex-linked lethal mutations in *Drosophila melanogaster* (fruit fly) is comparable to that of sulfur mustard (HD).

B. Estimated Reference Dose

Insufficient toxicity data are available to derive an RfD_e for agent T. Because agent T is a component of agent HT, its toxic effects may be similar to that of agent HT. However, the toxicity of agent HT is also poorly characterized, and quantitative dose–response data are not available. The lack of definitive toxicity data prevents derivation of a verifiable RfD_e for agent T.

VIII. Agent HN2 (Nitrogen Mustard)

A. Toxicology

Agent HN2 [bis(2-chloroethyl)methylamine] can cause nausea, vomiting, bone marrow suppression, and leukopenia, as well as hyperpigmentation of the skin and loss of hair and hearing (POISINDEX 1993). The chemical has been associated with birth defects in humans and is also a carcinogen.

1. Acute Toxicity. Symptoms of acute toxicity of agent HN2 include anorexia, weakness, drowsiness, headache, nausea, and vomiting (HSDB 1993). Reported oral LD_{50} values are 10–85 mg/kg for rats and 10–20 mg/kg for mice (Fox and Scott 1980; NDRC 1946a). When administered percutaneously, median lethality occurred at 12 and 14 mg/kg in rats, at 29–35 mg/kg in mice, and was estimated to be less than 50 mg/kg in monkeys (NDRC 1946a; Vojvodic et al. 1985). Intravenous LD_{50} values as low as 1.1 mg/kg in rats and approximately 2 mg/kg in mice have also been reported (Fox and Scott 1980; NDRC 1946a). In studies conducted by Conklin et al. (1965), FR mice given four intravenous injections of 2.4 mg HN2/kg at 14-d intervals exhibited reduced life span (490 d for animals surviving 30 d, compared to 632 d for controls). Decreased longevity was correlated with premature mortality from various diseases associated

with aging as well as with increased incidence of neoplasms. Premature development of lens opacities was also seen in the treated animals. In studies conducted on adult Carworth Farm mice, testicular lesions occurred in animals injected intraperitoneally with 4.0 or 6.3 mg/kg of agent HN2 (Landing et al. 1949a). An intraperitoneal injection of 8.4 mg/kg resulted in a significant decrease in white blood cell count ($p < .0045$) and mild pathological damage to the lymphoidal tissues, bone marrow, lungs, kidneys, gastrointestinal tract, and testes (Landing et al. 1949b).

2. Subchronic Toxicity. No studies were found in the available literature concerning the subchronic toxicity of HN2 in animals or humans.

3. Chronic Toxicity. No studies were found in the available literature concerning the chronic toxicity of HN2 in animals or humans by the oral exposure route. In a 50-wk study in which 20 mice were injected subcutaneously with 1 mg HN2 kg^{-1} wk^{-1}, 3 of 10 animals surviving for at least 250 d exhibited chronic lymphocytopenia (Boyland and Horning 1949).

4. Delayed Toxicity. Shimkin et al. (1966) reported testicular atrophy with decreased spermatogenic activity in A/J mice 39 wk after intraperitoneal treatment with HN2-hydrochloride. Twelve thrice weekly injections were given, resulting in total doses of 0.02, 1.1, 4.5, and 17.5 μmoles/kg.

5. Developmental and Reproductive Effects. Sokal and Lessmann (1960) reported that no abnormalities occurred in the offspring of four women with Hodgkin's disease who had been treated with HN2 during the first and third or second and third trimesters of pregnancy.

Nitrogen mustard and its hydrochloride salt have been shown to be teratogenic in mice and rats. In mice, a single intraperitoneal (i.p.) dose equivalent to 1 μg HN2-hydrochloride/g body weight, administered on the tenth to twelfth day of pregnancy, resulted in squat, edematous, exophthalmic embryos with practically no legs or tails (Danforth and Cater 1954). Haskin (1948) injected albino rats with 0.5 or 1 mg HN2-hydrochloride/kg subcutaneously (s.c.) on either day 12, 13, 14, or 15 of gestation, and all fetuses were removed at sacrifice on day 21. Six of nine litters of the surviving females contained fetuses with abnormalities. Effects were most severe in animals treated on day 12 of gestation. Abnormalities included receding lower jaws, cranial faults, cleft palate, deformed limbs, absence and fusion of digits, shortened tails, and unusual body proportions. Similar effects were reported by Murphy et al. (1958) for Wistar rats injected with HN2 on the twelfth day of gestation. Teratogenic effects, consisting primarily of syndactylous paws and cleft palate, were seen at a dose level as low as 0.5 mg/kg. Sanyal et al. (1981) reported that the embryonic development and organogenesis of rat embryos maintained in culture were significantly inhibited when HN2-hydrochloride, at concentrations of 1 or 5 μg/mL, was added to the culture medium.

6. Carcinogenic Effects. There is clinical evidence that patients treated with therapeutic doses of nitrogen mustards and other alkylating agents (as antineoplastic agents) for periods of weeks or months have an increased risk of developing acute nonlymphocytic leukemia (ANL) [see Institute of Medicine (1993) for review]. The peak time of onset of this leukemia is 3–9 yr after treatment, and the incidence rate is reported to be 3%–5% but may be as high as 30% in patients with prolonged or intensive treatment (Institute of Medicine 1993).

Animal studies have also demonstrated the carcinogenicity of HN2 and HN2-hydrochloride, as well as that of the related compound nitrogen mustard nitrogen-oxide hydrochloride. In a 50-wk study in which 20 mice were injected subcutaneously with 1 mg HN2 kg^{-1} wk^{-1}, 5 of 10 animals surviving for at least 250 d exhibited neoplasms of the lungs, including carcinomas in 3 animals, lymphosarcomas in 2, and an adenoma in 1 (Boyland and Horning 1949). In another study in which 0.1 mg HN2 (in 0.2 mL 95% ethanol) was applied to the shaved backs of female Swiss mice three times/wk, 9 of 33 exposed animals surviving for at least 112 d developed squamous cell carcinomas (Zackheim and Smuckler 1980). Similar results have been observed in other studies in which HN2 was administered by parenteral or dermal application [see IARC (1975) and Fox and Scott (1980) for reviews].

B. Estimated Reference Dose

In the derivation of an RfD, human exposure data are preferred; however, the only available data for HN2 pertain to acute exposures. Available animal data include acute toxicity data and short-term toxicity studies by parenteral routes of exposure. Acute toxicity endpoints are generally not used for developing subchronic or chronic reference values because they do not provide information on the possibility of cumulative effects following prolonged exposures. Thus, inadequacies in the available data base prevent the derivation of an RfD for HN2.

IX. Agent VX

Agent VX is an organophosphate cholinesterase (ChE) inhibitor similar to the G-agents in mode of action and toxic effects (DA 1974). VX volatility is very low (vapor pressure 0.0007 mm Hg) and droplets on the skin do not evaporate very quickly, thereby allowing extensive dermal absorption (DA 1974). By the dermal route, VX is more than 100 times as toxic as GB (DA 1974). The acute toxicity of VX and the G-agents has been reviewed in several earlier reports (Carnes and Watson 1989; DA 1974; Dacre 1984; Munro et al. 1994; O'Brien 1960; Sidell 1992; Somani et al. 1992; Watson et al. 1989b). This report focuses primarily on the available subchronic or chronic toxicity data that may be useful for deriving an oral RfD_o.

A. Toxicology

1. Acute Toxicity

Human Data. Limited information is available on the oral toxicity of VX to humans. In clinical studies conducted by Sidell and Groff (1974), single oral doses of 2–4.5 μg VX/kg produced gastrointestinal symptoms in 5 of 32 test subjects. Regression analysis of the dose–response data indicated that the RBC-ChE_{50} was 2.3 μg/kg. Sim et al. (1964) reported no signs of toxicity in 16 human volunteers receiving 1.43 μg VX kg^{-1} d^{-1} for 7 d (in four daily doses of 500 mL drinking water); however, average RBC-ChE activity was reduced 60% (to 40% of baseline values).

Data compiled by Sidell (1992) revealed that for individuals exposed to VX dermally, gastrointestinal symptoms (vomiting) occurred in 0.6% (1/166) when RBC-ChE activity was 50% of control values, and in 8% (2/24), 33% (9/27), 45% (19/42), and 67% (16/24) when RBC-ChE levels were 40%–49%, 30%–39%, 20%–29%, and less than 20% of control values, respectively. Sim (1962) reported that a dose of 5 μg VX/kg applied to the cheeks or ear lobes resulted in symptoms of systemic toxicity in about half the test subjects. Anxiety, psychomotor depression, intellectual impairment, and unusual dreaming occurred in volunteers whose RBC-AChE activity levels decreased to 41%–80% of baseline following application of droplets of EA-107 (agent VX) to various body sites (dose not given) (Bowers et al. 1964).

Several studies have been conducted in which human volunteers were injected intravenously with VX. Kimura et al. (1960) reported that a 30-sec intravenous injection of 0.04 μg/kg in one adult test subject caused headaches, tiredness, and irritability, but no change in RBC or whole blood ChE activity. A subsequent 30-sec intravenous injection of 0.08 μg/kg 3.5 hr later resulted in headaches, lightheadedness, and abdominal cramps as well as an increase in airway resistance, a decrease in respiratory rate, a decrease in pulse rate, and an increase in minute volume, but no change in ChE activity. A single 30-sec intravenous dose of 0.225 μg/kg in one test subject resulted in a 27% decrease in baseline RBC-ChE activity within 15 min and frontal retrobulbar headaches. Six subjects receiving 1 μg VX/kg by intravenous infusion for periods of 1.75–4 hr exhibited 50%–60% depression in ChE activity but no signs or symptoms of toxicity (except for one 84-kg individual who reported headaches). Sidell and Groff (1974) reported that an intravenous dose of 1.5 μg VX/kg in 18 test subjects resulted in dizziness, nausea, and vomiting in 11, 4, and 6 individuals, respectively; RBC-ChE was depressed 55%–90% from baseline values (average, about 75%). Regression analysis of dose– response data (doses of 1.2–1.7 μg/kg) indicated that the ChE_{50} was 1.1 μg VX/kg. Using the data provided by Kimura et al. (1960), McNamara et al. (1973) concluded that an intravenous dose of 0.1 μg/kg would have no effect on RBC-ChE activity.

Based on inhalation data for agent GB, McNamara et al. (1973) calculated the no-effect dose for VX-induced tremors in humans to be 0.34 μg/kg. Carnes

et al. (1986) suggested that the threshold for muscular tremors in sensitive subpopulations, such as infants, may be 0.16 μg/kg. McNamara et al. (1973) estimated that the human LD_{50} and no-death levels for VX were 7.5 μg/kg and 0.94 μg/kg, respectively. These estimates were based on extrapolations of LCt_{50} data for GB.

Animal Studies. The short-term toxicity of VX to Sprague-Dawley rats was investigated by Goldman et al. (1988). In one series of tests, a single subcutaneous injection of 1 μg VX/kg resulted in a 50% inhibition of RBC-AChE relative to controls, and a dose of 4 μg/kg resulted in a 87% inhibition relative to controls. In a second test series, male rats were dosed with 4 μg VX/kg by different exposure routes, and RBC-AChE levels relative to controls were determined at 3 hr and at 24 hr. High variability in response was seen in animals dosed by intratracheal instillation, intragastric lavage, and intraperitoneal injection. RBC-AChE levels were reduced a similar amount for subcutaneous injections (13% ± 7% of control values at 3 hr and 21% at 24 hr) and intravenous injections (14% ± 0.07% of control values at 3 hr and 20% at 24 hr). In another pilot study, male and female rats (8–10 wk old) were injected subcutaneously with 0, 0.25, 0.63, 1.56, 3.91, 9.77, or 14.65 μg VX kg^{-1} d^{-1}, 5 d/wk, for 14 d. Each dose group consisted of eight males and eight females except for the high-dose group, which had two males and two females. All the animals in the two highest dose groups died as a result of the exposures, but none of the animals in the three other dose groups died as a result of the exposures. Of the remaining animals, one-half were killed at 7 d and the other half at 14 d. RBC-AChE activity levels were depressed in all dose groups in a dose–dependent manner. Results for dose levels of 0.25 and 1.56 μg kg^{-1} d^{-1} are included in Table 14).

2. *Subchronic Toxicity.* The subchronic toxicity of VX to Sprague-Dawley rats was investigated by Goldman et al. (1988), who injected male and female animals (25 of each gender/dose group) subcutaneously with VX once per day, 5 d/wk, for up to 90 d. The administered doses were 0 (saline controls), 0.25, 1.0, or 4.0 μg VX kg^{-1} d^{-1}. Five animals of each gender were killed at 30, 60, and 120 d (includes a 30-d recovery period), and 10 animals of each gender were killed at 90 d. RBC-AChE activity levels were monitored in 2 of 5 or in 3 of 10 animals per gender at each of the sacrifice times; blood chemistry was evaluated in the remaining animals. The tissues of all sacrificed animals were processed for histological analysis. Urinalyses were conducted on samples collected during wk 8 and 12. Animals in the highest dose group exhibited body weight loss and behavioral changes (increased irritability and aggressiveness by wk 2, followed by decreased grooming and lethargy at wk 8). There were periodic cases of diarrhea in this group. By wk 5, some of the animals dosed with 1.0 μg kg^{-1} d^{-1} exhibited some irritability.

Relative brain weight (ratio of brain to body weight) was elevated in the 4.0 μg kg^{-1} d^{-1} group. There were no significant changes in clinical chemistry or urinalysis parameters that were dose related. Histopathological examination did

Table 14. RBC-ChE activity in rats injected subcutaneously with agent VX.[a]

Time of sacrifice	Sex	No.	VX dose (μg kg^{-1} d^{-1})[b]			
			0.25	1.0	1.56	4.0
7 d[c]	M	4	0.85 ± 0.06	—	0.46 ± 0.07	0.31 ± 0.03[d]
	F	4	0.90 ± 0.05	—	0.36 ± 0.09	0.34 ± 0.13[d]
	M	4	0.72 ± 0.07	—	0.34 ± 0.12	0.29 ± 0.03[d]
14 d[c]	F	4	0.64 ± 0.12	—	0.28 ± 0.13	0.31 ± 0.08[d]
	M	2	0.46 ± 0.04[g]	0.22 ± 0.00[g]	—	0.04 ± 0.02[g]
30 d[e]	F	2	0.48 ± 0.06[g]	0.20 ± 0.01[g]	—	0.10 ± 0.05[g]
	M	2	0.33 ± 0.17	0.23 ± 0.06	—	0.14 ± 0.00
60 d[e]	F	2	0.53 ± 0.04	0.34 ± 0.02	—	0.23 ± 0.02
	M	3	0.34 ± 0.02	0.37 ± 0.03	—	0.23 ± 0.08
90 d[e]	F	3	0.64 ± 0.13	0.51 ± 0.17	—	0.27 ± 0.07
	M	5	0.91 ± 0.07	0.88 ± 0.11	—	0.83 ± 0.11
120 d[f]	F	5	0.98 ± 0.12	0.93 ± 0.13	—	0.88 ± 0.16

[a]Data expressed as fraction of control value; mean ± SD; includes data from 14-d pilot study and 90-d study.

[b]Dosing schedule: 1/d, 5 d/wk.

[c]Fourteen-day study.

[d]Dose was 3.91 μg kg^{-1} d^{-1}.

[e]Ninety-day subchronic study.

[f]Includes recovery period of 30 d.

[g]Analyzed statistically by ORNL, and found to be significantly ($p < .05$) lower then control values (Scheffe's and Dunnett's comparisons). Other exposure periods were not analyzed statistically.

Source: Goldman et al. (1988).

not indicate a VX-associated pathology. In a separate three-generation study in which hematological parameters were evaluated in rats maintained for 120 d under the same exposure protocol, there were no significant effects in male rats; however, in F_0 females dosed with 4.0 μg kg^{-1} d^{-1}, statistically significant decreases occurred in hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin (Goldman et al. 1988).

Plasma ChE was significantly reduced at 30 d ($p < .05$) in males and females given 1.0 μg kg^{-1} d^{-1} and at 30, 60, and 90 d for males and females given 4.0 μg kg^{-1} d^{-1}. RBC-AChE levels in males and females were reduced in a dose-dependent manner when compared to control values for the same time period (see Table 14); however, the study did not include baseline or preexposure RBC-AChE levels for each test group. Goldman et al. (1988) reported that the observed decreases were significant but the level of statistical significance was not reported. The 30-d data were reanalyzed using analysis of variance (ANOVA) and Dunnett's and Scheffe's comparisons, and RBC-AChE activity in all dose groups for both males and females was significantly lower ($p < .05$) than control values for the same time period. RBC-AChE levels in both males and females

returned to 83%–98% of control values in the test groups allowed a 30-d recovery period. Daily exposure to VX by subcutaneous injection for 30 d resulted in statistically significant depression of RBC-AChE at all dose levels.

Rice et al. (1971) conducted toxicity studies in which sheep (Columbian, or Columbian crossed with Shropshire or Rambouillet) were fed VX once or three times per day for varying exposure durations. In one phase of this study, healthy yearling ewes were given daily VX doses of 0, 3, 9, or 15 μg/d (5 animals per dose group and 10 controls) for 56 d. The agent was mixed with Pillsbury 16% rabbit pellets and hand-fed to the test animals. The animals were checked periodically during the feeding period for clinical signs of toxicity (i.e., slowed pupil response, slowed pain reflex, and profuse salivation). There were no reported data identifying changes in clinical chemistry (except whole blood ChE), hematology, body and organ weights, or gross or microscopic pathology. Whole blood ChE activity levels were monitored before the first exposure (one determination per animal) and then 16 times during the 56-d test period. Each whole blood ChE determination was first normalized to the average control value for the same time period to give an adjusted daily value. The normalized values for each time period were then compared to the average normalized preexposure (baseline) ChE value for each dose group (Table 15). Whole blood ChE was significantly depressed at all dose levels (levels of significance not reported); at the lowest dose (3 μg/d), the decrease in ChE was statistically significant by the twenty-first day, then became stabilized at about 62% of the baseline by the thirty-first day and remained at this level for the remainder of the 56-d feeding period (Figure 1).

The sheep used in this study had an average weight of 52.7 kg; therefore, the weight-normalized dose for 3 μg/d was 0.06 μg kg^{-1} d^{-1}. None of the sheep dosed with 3, 9, or 15 μg/d exhibited any physical signs of clinical toxicity,

Table 15. RBC-ChE activity in sheep fed agent VX.[a]

	3 μg VX/d		9 μg VX/d		15 μg VX/d	
Time (hr)[a]	Fraction control[b]	Fraction baseline[c]	Fraction control[b]	Fraction baseline[c]	Fraction control[b]	Fraction baseline[c]
0	1.15	1.00	1.03	1.00	1.17	1.00
24	1.05	0.91	0.84	0.81	0.86	0.74
240	1.16	1.01	0.58	0.57	0.51	0.44
360	0.92	0.80	0.55	0.53	0.29	0.24
744	0.72	0.62	0.26	0.25	0.09	0.07
912	0.73	0.64	0.12	0.11	0.05	0.04
1320	0.72	0.63	0.20	0.19	0.08	0.07

[a]Selected data points presented.
[b]Data expressed as a fraction of the control value ChE.
[c]Data expressed as fraction of adjusted baseline ChE.
Source: Rice et al. (1971).

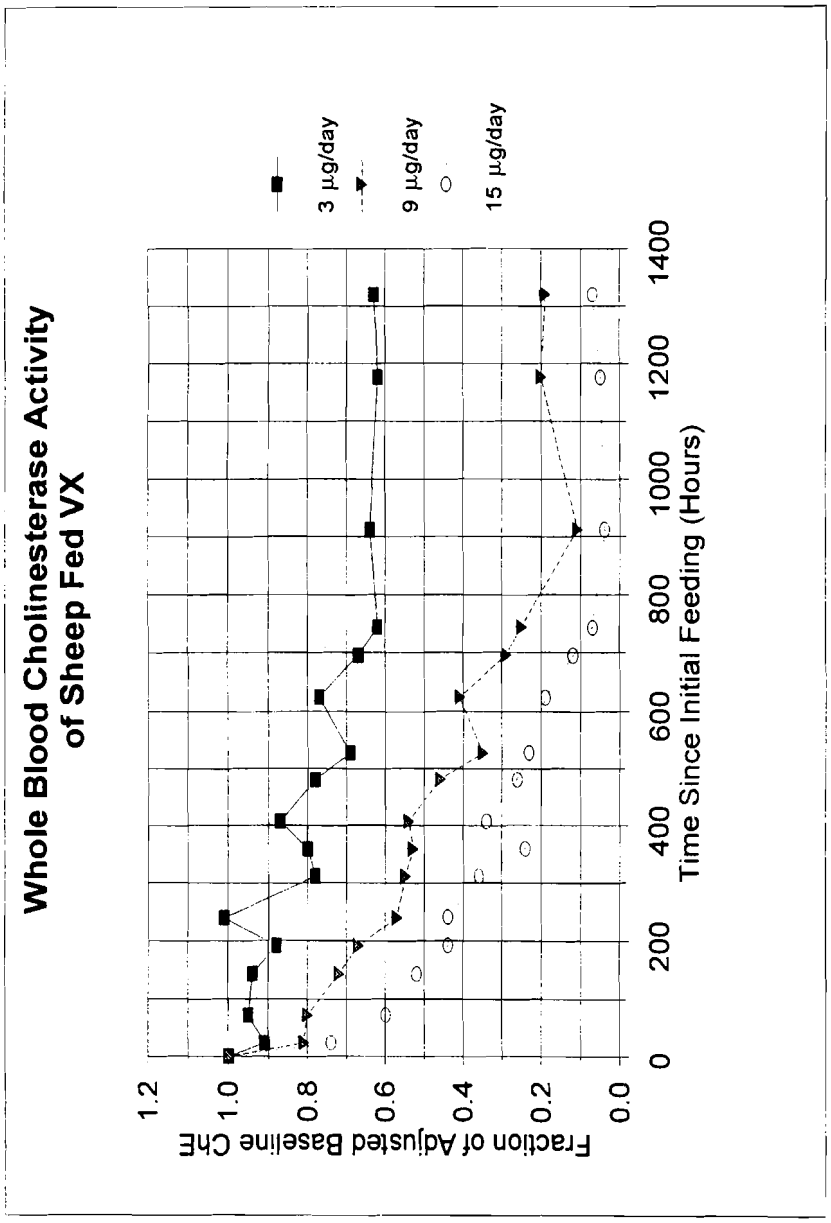


Fig. 1. Effects of agent VX on blood cholinesterase activity levels in sheep (adapted from Rice et al. 1971).

even though the whole blood ChE in the highest dose group was reduced to 5% of the normalized baseline values for the last 3 wk of the feeding period. In other phases of the Rice et al. (1971) study, physical signs of toxicity (not described in detail) were observed in "culled" or weakened ewes dosed with 30 μg VX/d for about 4 wk and in healthy sheep dosed with 75 μg VX/d for about 3 wk. Rice et al. (1971) noted that the culled animals surviving the exposure recovered fully without developing any permanent signs of toxicity. Because of the significant reduction in whole blood ChE (38% relative to preexposure values), the lowest test dose (3 μg/d) in the 56-d study is considered a lowest-observed-adverse-effect level (LOAEL). It should be noted, however, that a LOAEL for physical signs of clinical toxicity occurred at 75 μg/d in the 3-wk study, and the highest dose level at which no clinical signs occurred was 15 μg/d in the 56-d study. In the latter case blood ChE activity was reduced to 4% of the preexposure value by d 38.

4. Chronic Toxicity. Data on the toxicity of agent VX to humans or animals following long-term exposures were not found in the available literature.

5. Neurotoxicity. Sidell and Groff (1974) reported that volunteers dosed with VX (1.5 μg/kg, intravenously) exhibited a significant decrement in performance on a number facility test within 1 hr after treatment. Bowers et al. (1964) reported anxiety, psychomotor depression, intellectual impairment, and unusual dreaming in volunteers exposed to droplets of VX on their skin and in whom RBC-AChE activity was subsequently depressed 70% or more.

No clinical or experimental evidence is available to indicate that VX causes delayed neuropathy in humans [see Munro et al. (1994) for review]. Gordon et al. (1983) found that VX had a very low potential for inhibition of neuropathy target esterase (NTE) when tested *in vitro* on brain tissue from chickens. Delayed neuropathy was not observed in three strains of antidote-protected chickens given a single subcutaneous dose of VX as large as 0.15 mg/kg (estimated to be 5–10 times the lethal level), and repeated intramuscular injections of VX (0.04 mg kg^{-1} d^{-1}, 3d/wk for 30 d, or 5 d/wk for 90 d) also did not produce any signs of organophosphate-induced delayed neuropathy (OPIDN) (Goldman et al. 1988; Wilson et al. 1988). In comparison, the LD_{50} value for an intramuscular injection of VX in chickens is about 0.03 mg/kg (Goldman et al. 1988). In 90-d subchronic studies conducted on Sprague-Dawley rats, Goldman et al. (1988) found no incidences of tissue degeneration in brain, spinal cord, or peripheral nerves that could be associated with daily subcutaneous injections of as much as 4 μg VX/kg for 5 d/wk. Although *O*-ethyl-*O'*-(2-diisopropylaminoethyl)methylphosphonite (QL), a chemical intermediate of VX, has been reported to cause delayed neurotoxic effects in hens dosed at 635 mg/kg or more (Olajos et al. 1986), the overall data indicate that delayed neuropathy is unlikely to occur in humans exposed to agent VX.

6. Developmental and Reproductive Effects. The effects of VX on the development and reproduction of sheep were evaluated by Van Kampen et al. (1970) following an accidental release of the nerve agent VX in Skull Valley, UT. Of some 6300 affected animals, about 4500 died or were killed (Van Kampen et al. 1970). When 79 surviving animals that had been pregnant at the time of exposure and their lambs were evaluated for changes in RBC-AChE activity and for signs of toxicity over a 6-mon postexposure period, RBC-AChE activity in the ewes remained significantly depressed for about 4 mon and then returned to normal. Ewes that were killed at 2-wk intervals had no gross or microscopic evidence of damage to the central nervous system. Torticollis (wryneck) developed in 1 ewe 1 wk following exposure and persisted for 9 mon (a similar effect was seen in 1 of 38 ewes dosed in the laboratory with an undisclosed amount of VX). Of the lambs born 2–3 mon after exposure of the ewes, only one (total number examined not reported) exhibited deformities (extra oral opening below the right ear); but these malformations were not considered related to agent exposure. None of the lambs displayed neurotoxic signs or symptoms, and their whole blood ChE activity was not reduced even when suckling from exposed and affected ewes. Five months after exposure, the ewes exposed in the field as well as ewes dosed with an undisclosed amount of VX 4 mon previously were mated to unexposed males. Examination 4 mon later indicated that fetal growth and development were normal except for one fetus that appeared stunted (total number examined not reported). The investigators concluded that VX had little or no effect on fetal growth or development.

Goldman et al. (1988) administered VX subcutaneously to Sprague-Dawley rats on d 6–15 of gestation at 0, 0.25, 1.0, or 4.0 μg kg^{-1} d^{-1}. Body weight, frequency of visceral and skeletal abnormalities, litter size, and sex ratios were evaluated. The examined fetuses showed no evidence of malformations. Fetal body weight, litter size, and sex ratio were within normal limits. Blood ChE activity levels were not monitored.

Goldman et al. (1988) administered subcutaneous doses of 0, 0.25, 1.0, or 4.0 μg VX/kg to New Zealand white rabbits on d 6–19 of gestation. Animals were also observed daily for signs of toxicity. The does were killed on d 29 of gestation. Body weight, fetal weights, fetal deaths, frequency of visceral and skeletal abnormalities, litter size, and sex ratios were evaluated. There was no statistical evidence that VX affected any of the parameters studied. Blood ChE activity levels were monitored in a 7-d pilot study that also included a dose of 8 μg/kg. The 8 μg/kg dose was severely toxic to the rabbits (1/3 died, 2/3 were ataxic). The dose of 0.25 μg/kg resulted in a level of RBC-AChE inhibition equal to 0.71 of the control value, but produced no signs of toxicity.

In a modified dominant lethal study, Goldman et al. (1988) administered VX by subcutaneous injection to male or female Sprague-Dawley rats and observed the effects on various parameters including terminal body weight, testes weight, testicular histopathology, maternal weight, implantation sites, resorptions, and total corpora lutea. The test animals were dosed with 0 (saline control), 0.25, 1.0, or 4 μg VX kg^{-1} d^{-1} for 10 wk. Triethylenemelamine was used as a positive

control. Exposure to VX produced no significant changes in body or organ weights. VX had no adverse effects on preimplantation losses as evaluated by number of implants, live fetuses, dead fetuses, and resorptions. Microscopic examination of the testes did not reveal any abnormalities that could be attributed to VX exposure.

In a three-generation study, male and female Sprague-Dawley rats were dosed subcutaneously with 0 (saline controls), 0.25, 1.0, or 4.0 μg VX kg^{-1} d^{-1}, 5 d/wk (Goldman et al. 1988). The F_0 generation (11–12 males and 24 females per dose group) was dosed for about 105 d, after which period they were mated, and the dosing continued through gestation and weaning (total duration of dosing, 21–25 wk). Dosing of the F_1 generation began after weaning and continued for approximately 126 d, after which they were mated, and dosing continued through gestation and weaning (total duration, 24–27 wk). Five males and 5 females of each dose group of the F_2 generation were killed at weaning. The study included analysis of pup mortality in each of the generations, body and organ weight changes and hematological parameters in the F_0 generation, and histopathological examination of tissues (including nervous system, reproductive system, gastrointestinal tract, lung, liver, and kidney) of the F_1 parental males and females, the F_1 weanlings, and the F_2 weanlings. Blood ChE activity levels were not monitored during the study.

VX exposure had no adverse effect on the number of pups born in the F_1 or F_2 generation. Perinatal mortality (i.e., percent of pups born dead or dying within 24 hr of birth) was not significantly different among dose levels for both generations; however, perinatal mortality in the high-dose group (5.7%) was considerably higher than that in the lower-dose groups (1.2%). Pup mortality from birth to weaning was significantly ($p < .01$) related to VX exposure primarily for the F_1 generation pups in the 4.0 μg kg^{-1} d^{-1} dose group. Goldman et al. (1988) attributed this increase to the effect of VX on the dams, which resulted in the increased incidence of cannibalism of the pups; the investigators concluded that under the conditions of the test, there was no evidence of direct VX reproductive toxicity. The hematological studies conducted on dosed males of the F_0 generation revealed no significant VX-associated effects. In females dosed with 4.0 μg VX kg^{-1} d^{-1}, statistically significant decreases occurred in hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin. Body and organ weight analysis and histopathological examination revealed three effects that may have been dose related: changes in brain weight, incidence of eosinophilic gastritis, and incidence of pituitary cysts. However, Goldman et al. (1988) attributed the first two effects to statistical chance and considered the third as not being biologically significant. The overall conclusion of the investigators was that there were no organ weight or microscopic changes that could be attributed specifically to the action of VX.

7. Carcinogenicity. No information is available regarding the potential carcinogenicity of VX in humans. Standard long-term carcinogenicity studies have not been conducted on laboratory animals exposed to VX. Neoplastic lesions

were not observed in male and female Sprague-Dawley rats injected subcutaneously with as much as 0.25, 1.0, or 4.0 μg VX kg^{-1} d^{-1} for 90 d (Goldman et al. 1988). No other animal data are available to assess the potential carcinogenicity of VX.

8. Genotoxicity. No information is available regarding the genotoxicity of VX in humans. In tests on microorganisms and mammalian cell cultures, VX was not found to be mutagenic or was only weakly mutagenic (Goldman et al. 1988). VX did not induce biologically significant increases in mutations when tested in the Ames *Salmonella* assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) with and without metabolic activation (Goldman et al. 1988). In tests using the yeast *Saccharomyces cerevisiae*, VX did not induce recombinants following exposures to concentrations as high as 100 μg/mL (Goldman et al. 1988). VX also failed to induce forward mutations when tested on mouse L5178Y lymphoma cells at concentrations less than 50 μg/mL (Goldman et al. 1988). Doses of 50 and 100 μg VX/mL resulted in increased numbers of mutations, but these were not more than 1.5 times the control level (a 2-fold increase was considered the minimum required to establish a positive result).

B. Estimated Reference Dose

1. Selection of the Key Study. No chronic animal toxicity studies have been conducted on VX; however, there are two subchronic studies that can be used for developing an RfD. In one study, rats were dosed by subcutaneous injection 5 d/wk for 90 d (Goldman et al. 1988). In the second study, sheep received daily doses of VX in feed (Rice et al. 1971). Both of these studies identify blood ChE as the most sensitive endpoint. Data are available indicating that sheep are more sensitive than rats to the toxic effects of VX. Ivanov et al. (1993) reported that the oral LD_{50} in sheep is 6 μg/kg whereas that for rats is 66 μg/kg. In addition, Ivanov et al. (1993) suggested that this increased susceptibility in sheep may result, in part, from the lower concentration of catalytic sites for serum ChE in sheep (7.098×10^{-10} mol/L vs. 1.704×10^{-9} mol/L in rats). The study by Rice et al. (1971) is selected here for deriving an oral RfD because it utilized an exposure route that is more relevant for an oral RfD, and also because the experimental evidence indicates that sheep are the more sensitive of the two species tested.

In one phase of the Rice et al. (1971) study, healthy yearling ewes were fed agent VX for 56 d. Changes in whole blood ChE activity were measured over this time period. The dose levels were 0, 3, 9, and 15 μg VX/d. Whole blood ChE was significantly depressed at all dose levels (levels of significance not reported) without any physical signs of clinical toxicity in any dose group. At the lowest dose (3 μg/d, equivalent to 0.06 μg kg^{-1} d^{-1}, based on the reported average body weight of 52.7 kg), the decrease in whole blood ChE was statistically significant by the twenty-first day. Because of this significant reduction in ChE (38% relative to preexposure values), this low dose is considered an effect

level. However, it should be pointed out that this response, in the absence of clinical signs, may only represent a biological marker of exposure rather than a sign of adverse physiological change, and for that reason it is considered here to be a minimal LOAEL (see Section III for further discussion of the use of ChE inhibition as a critical effect). In other phases of the Rice et al. (1971) study, the lowest dose level at which physical signs of toxicity were observed was 30 μg/d for about 4 wk in "culled" or weakened ewes and 75 μg/d for about 3 wk in healthy sheep; therefore, these higher exposure levels could be considered true LOAELs. The highest dose level at which no clinical signs of toxicity occurred was 15 μg/d.

2. RfD_c Derivation. The LOAEL of 0.06 μg kg^{-1} d^{-1} derived from the Rice et al. (1971) study is used to estimate a human oral reference dose (RfD_c) by using the following formula:

$$RfD_c = \frac{\text{LOAEL } (\mu g/kg/d)}{UF_H \times UF_A \times UF_S \times UF_L \times UF_D \times MF}$$

where:

LOAEL = 0.06 μg kg^{-1} d^{-1}
UF_H = 10 (sensitive subpopulations)
UF_A = 1 (animal to human extrapolation)
UF_S = 3 (extrapolation from subchronic to chronic exposures)
UF_L = 3 (LOAEL to a NOAEL extrapolation)
UF_D = 1 (data base completeness)
MF = 1 (Modifying factor)

A UF_H of 10 for sensitive subpopulations is considered necessary because some individuals have abnormally low levels of blood ChE activity that may make them especially susceptible to the effects of ChE inhibitors such as nerve agents (see Section III.B.6 for additional discussion).

An uncertainty factor is not used to extrapolate from animals to humans because there is sufficient evidence that humans are not more sensitive to VX than sheep. The following evidence is available to support this position:

1. Sheep have a much lower RBC-AChE activity level compared to humans, 2.9 μmol mL^{-1} min^{-1} vs. 12.6 μmol mL^{-1} min^{-1} (Ellin 1981). If ChE activity in the blood acts as a buffer to the effects of anticholinesterase compounds, than the lower activity level in sheep may cause them to have a higher susceptibility to agents such as VX.
2. In humans, a daily oral dose of 1.43 μg/kg for 7 d resulted in a 60% reduction in RBC-AChE activity (Sim et al. 1964), whereas in sheep a nearly equivalent reduction in whole blood ChE (56% inhibition) resulted from a dose of only 0.28 μg kg^{-1} d^{-1} administered for 8–13 d (Rice et al. 1971). In sheep, daily doses as low as 1.1 μg/kg (divided into three equal doses/d) resulted in a 75% reduction in blood ChE in 6 d, an 83% reduction in 10 d, and also produced

physical signs of toxicity (Rice et al. 1971). [Note: in sheep, about 90% of the blood ChE activity is in the RBC fraction (Osweiler et al. 1985); therefore, sheep whole blood ChE measurements can be reasonably compared to human RBC-AChE values.]

3. The whole blood ChE_{50} in sheep is about 2.4 μg VX/kg (estimated from data presented by Rice et al. 1971), and the oral RBC-$AChE_{50}$ in humans is about 2.3 μg/kg (Sidell and Groff 1974).
4. The similarities in VX sensitivity between sheep and humans may, in part, be accounted for by similarities in rates of metabolic detoxification of the agent. The latter can be estimated from a comparison of body surface areas (based on body weight) as is done for animal-to-human extrapolations used in EPA cancer risk assessments (USEPA 1996c). Using this approach, the human equivalent dose for the 0.06 μg/kg/d sheep LOAEL in the Rice et al. study can be calculated as:

$$LOAEL_{human} = LOAEL_{sheep} \times \frac{1}{\left(\frac{BW_{human}}{BW_{sheep}}\right)^{0.25}}$$

where:

Bw_{human} = default body weight of 70 kg for humans
BW_{sheep} = average body weight of 52.7 kg for sheep in the study by Rice et al. (1971)
$LOAEL_{sheep}$ = the experimental dose of 0.06 μg kg^{-1} d^{-1} for sheep

therefore:

$$LOAEL_{human} = 0.06\ \mu g/kg/d \times \frac{1}{\left(\frac{70\ kg}{52.7\ kg}\right)^{0.25}} = 0.056\ \mu g/kg/d$$

This calculation indicates that the human dose to produce a similar level of effect would not be substantially different from that in sheep. Considered together with the blood ChE activity and toxicity values mentioned previously, the evidence is considered sufficient to support the use of an Uncertainty Factor of 1 for animal-to-human extrapolation.

An uncertainty factor of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of oral RfDs for other organophosphate compounds, EPA has used NOAELs for ChE inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a subchronic-to-chronic UF of 1 was used). In addition, animal data indicate that maximum ChE inhibition may occur 30–60 d or more after exposure begins, after which it levels off or even shows signs of recovery. This pattern can be seen in the data for the Rice et al. (1971) study (see Fig. 1). However, an uncertainty factor of 3 is used

here because chronic studies are not available to verify the unlikelihood that additional effects would occur following chronic exposures.

A LOAEL-to-NOAEL uncertainty factor of 3 is used instead of 10 because the endpoint, ChE inhibition, was not associated with any reported physical signs of clinical toxicity. Furthermore, regression analysis of the Rice et al. (1971) data (Table 16) indicates that 30% inhibition of ChE, which is considered to be the threshold for a biologically significant level of inhibition by EPA (personal communication, H. Choudhury, 1996), would have occurred at about 2 μg/d, substantially above the NOAEL value of 0.3 μg/d that would result from applying a full UF_L of 10 to the LOAEL of 3 μg/d. Furthermore, as noted, the lowest dose level at which physical signs of toxicity occurred was 30 μg/d, about 10 times higher than the dose level used to derive the RfD_c.

The data base requirements have been met in that there are subchronic toxicity studies in two species (sheep and rats), teratology studies in two species (rats and rabbits), a modified dominant lethal study in rats, a delayed neuropathy study in chickens, and a multigeneration study in rats. In addition, there are substantial human data supporting the RfD. The uncertainty associated with the absence of a chronic toxicity study is accounted for in UF_S. No modifying factor is required in the derivation of the RfD for VX.

The estimated oral RfD_c for VX is therefore:

$$RfD_c = \frac{0.06\ \mu g/kg/d}{10 \times 1 \times 3 \times 3 \times 1 \times 1}$$

$$RfD_c = 0.0006\ \mu g\ VX/kg/d$$

Table 16. Regression analysis of data from Rice et al. (1971) for sheep dosed with agent VX.

	Linear analysis		Log transformation	
Time (hr)[a]	Mean dose[b] for 30% ChE inhibition (μg/d)	r^2	Mean dose[b] for 30% ChE inhibition (μg/d)	r^2
744	0.88	0.961742	1.94	0.993047
912	1.69	0.836172	2.26	0.940522
1176	0.97	0.930412	1.97	0.980956
1320	1.21	0.90184	2.08	0.969214
Overall mean[c]	1.33		2.15	
SD	0.46		0.24	
Lower 95% CL	0.76		1.86	
Upper 95% CL	1.90		2.45	

[a]Data analysis based on four time points at which ChE inhibition stabilized.
[b]Based on %ChE inhibition at 3, 9, and 15 μg/d (see Fig. 1).
[c]Derived from means for 744, 912, 1176, and 1320 hr.

3. Overall Confidence in the RfD_e.

Study: Medium
Data base: High
RfD: High

The database for VX consists of subchronic studies in sheep and rats, teratology studies in rats and rabbits, a modified dominant lethal study in rats, a delayed neuropathy study in chickens, and a multigeneration study in rats. In addition, there are also data available evaluating the effects of VX in humans following acute and short-term exposures. Although the principal study did not report on clinical chemistry, hematology, body and organ weight changes, or gross or histological pathology, there are supporting studies to indicate that ChE inhibition is the appropriate endpoint. There is also evidence that sheep are one of the most sensitive species in their response to ChE inhibitors. Therefore, the overall confidence in the RfD_e is high.

4. Comparison of RfD_e with Human Toxicity Data. The proposed RfD_e is compared to the available human toxicity data in Table 17. One study in humans indicated that an oral dose of 1.43 μg kg^{-1} d^{-1} for 7 d resulted in a 60% RBC-AChE inhibition but no toxic effects (Sim et al. 1964). This dose is more than 2000 times greater than the RfD_e. For an adverse-effect level (i.e., mild toxic effect at 2–4.5 μg kg^{-1} d^{-1}), the "margin of safety" would be larger. The results of the study by Sim et al. (1964), indicating a LOAEL of 1.43 μg kg^{-1} d^{-1} for a 7-d exposure, were used to calculate an oral RfD_e for comparison with the RfD_e derived from the animal data. Using a UF_H of 10 for sensitive subpopulations, a UF_L of 10 for a LOAEL-to-NOAEL extrapolation (10 is chosen because the dose of 1.43 μg kg^{-1} d^{-1} produced a 60% inhibition of RBC-ChE, which could be close to the toxic effect level), and a UF of 3 for protecting against longer exposures (animal data indicate that maximum ChE inhibition may occur 30–60 d after exposure begins and the Sim et al. study was for only 7 d), the resulting estimated oral RfD is 0.005 μg kg^{-1} d^{-1}, a value approximately one order of magnitude greater than the RfD_e of 0.0006 μg kg^{-1} d^{-1} estimated from the Rice et al. (1971) sheep data.

X. Agent GA (Tabun)

Agent GA (tabun) is an organophosphate ChE inhibitor similar to other nerve agents in mode of action and toxic effects, and it is toxic by all possible exposure routes: ingestion, inhalation, and ocular and percutaneous absorption (DA 1974). By the inhalation exposure route, GA is only half as toxic as GB; however, at low concentrations it has a greater effect on the eyes (DA 1974). The acute toxicity of GA and other nerve agents has been reviewed in several earlier reports (Carnes and Watson 1989; Dacre 1984; Munro et al. 1994; Sidell 1992;

Table 17. Comparison of RfD_c with human toxicity data for agent VX.

Dose (μg/kg)	Exposure route	Endpoint	References
0.0006[a]	Oral	RfD_c: no inhibition of RBC-ChE	This report
0.1	Intravenous	Estimated no-effect level for RBC-ChE inhibition	McNamara et al. (1973)
0.24	Oral	Estimated no-effect level for RBC-ChE inhibition, based on ratio of oral to intravenous doses required for 50% RBC-ChE inhibition	This report
0.34	Inhalation	Estimated threshold for tremors based on inhalation data for GB	McNamara et al. (1973)
1.0	Intravenous	50% inhibition of RBC-ChE	Sidell and Groff (1974)
1.43[b]	Oral	60% inhibition of RBC-ChE; no signs or symptoms of toxicity	Sim et al. (1964)
2.4	Oral	50% inhibition of RBC-ChE	Sidell and Groff (1974)
2–4.5	Oral	Gastrointestinal symptoms in 5/32	Sidell and Groff (1974)

[a]Daily dose for chronic exposure period.
[b]Administered in four equally divided doses/d in 500 mL water, for 7 d.

Somani et al. 1992; Watson et al. 1989b). This review focuses primarily on the available subchronic or chronic toxicity data that may be useful for deriving an oral RfD_c.

A. Toxicology

1. Acute Toxicity. GA lethality data for animals and estimates of human LD_{50} values are given in Table 18. Oral LD_{50} values for humans have been estimated to be 357–714 μg/kg (Somani et al. 1992). A subcutaneous injection of 0.43 μmol/kg (0.0698 mg/kg) in dogs resulted in a depression in erythrocyte ChE activity to about 30% of its baseline value; however, no overt toxic effects were observed (Holmstedt 1951). In cats, a slow drip intravenous infusion of 0.05 mg/kg or more resulted in increased bronchial constriction and labored respiration (Holmstedt 1951). In rabbits, an intravenous dose of about 0.04 mg/kg (25% of the lethal dose) caused a steady decrease in cardiac output (Holmstedt 1951).

2. Subchronic Toxicity. The National Center for Toxicological Research (NCTR) evaluated the subchronic toxicity of agent GA on male and female NCTR cesarian-derived Sprague-Dawley (CD) rats (Bucci et al. 1992a). The test animals (12 of either sex/dose group) were injected intraperitoneally with GA at dose levels equivalent to 0, 28.13, 56.25, or 112.5 μg/kg. The injections

Table 18. LD_{50} values for agent GA.

Exposure route	Species[a]	LD_{50} (μg/kg)	References
Intravenous	Human	14[b]	Robinson (1967)
	Monkey	~50	DA (1974)
	Rat	70	DA (1974)
Oral	Human	357–714[c]	Somani et al. (1992)
	Rat	3,700	Wilson and Sondheimer (1957)
Subcutaneous	Monkey	70	RTECS (1995a)
	Rat	162	RTECS (1995a)
	Rat	~30	DA (1974)
Percutaneous	Human	14,000–21,000	DA (1974)
	Human	2,857–14,286[c]	Somani et al. (1992)
	Monkey	9,300	RTECS (1995a)
	Rat	18,000	RTECS (1995a)
	Rat	12,600	Crook et al. (1983)
Intramuscular	Monkey	34	RTECS (1995a)
	Rat	800	RTECS (1995a)
Intraperitoneal	Rat	~800	DA (1974)
		490	RTECS (1995a)

[a]Values for humans estimated from animal data.
[b]LD_{Lo}.
[c]Estimated for 70-kg individuals.

were given once per day, 5 d/wk, for 13 wk. Animals were observed daily for clinical signs of toxicity. Necropsy examination was performed on all animals. Terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals as well as on all gross lesions and on animals dying or killed before the end of the test period. Blood samples were taken from 6 rats per sex/dose during wk −1, 1, 3, 7, and at necropsy. Hematological analyses consisted of blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, hemoglobin, and hemoglobin concentration. Clinical chemistry included measurements of alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, creatinine kinase, and RBC- and plasma-ChE. In addition, at necropsy, brain samples were tested for NTE.

The results of the clinical chemistry tests indicated no adverse effects on liver, kidney, or muscle. Hematological parameters for the dosed animals were generally within the normal range, and brain NTE activity was not affected by GA administration. There were no GA-related neoplastic or nonneoplastic lesions.

ChE activity levels in the dosed rats were compared to control values for the same sampling times. There was considerable variability in the RBC-AChE data (Table 19). Mean baseline values for both male and female rats were elevated substantially (5029 vs. 1848 IU/L in females and 4045 vs. 1552 IU/L in males) when compared to control levels recorded in previous nerve agent studies conducted at the same laboratory. The elevated preexposure RBC-AChE readings in the current study were attributed to faulty reagents. Mean RBC-AChE activity levels in dosed and concurrent control animals (0 μg GA kg^{-1} d^{-1}) also showed irregular fluctuations over time, with unusually high readings occurring in females at wk 3 and in males at wk 7. It was reported that the percent reduction in RBC-AChE at wk 3 was about 37% in dosed females and 18% in males. Statistical analysis of the RBC-AChE data indicated significant reductions in RBC-AChE activity (relative to controls) in only a few cases; i.e., in high-dose females at wk 3; in mid-dose females at wk 7; and in males of all dose groups at wk 1. The RBC-AChE data were reanalyzed by ORNL with ANOVA, the *t*-test, and Dunnett's and Scheffe's comparisons. This analysis indicated that RBC-AChE levels in males at wk 1 were not significantly lower than control values (Scheffe's and Dunnett's comparison), but were significantly lower than preexposure values ($p < .05$, Dunnett's, but not Scheffe's) for the two lowest dose groups but not the high-dose group. The *t*-test indicated significant differences from preexposure values at wk 1 for all dose levels. Similar reanalysis of the female RBC-AChE data indicated that the high-dose group had significant reductions from preexposure values at wk 1, 3, and 7 and significant reductions from controls at wk 3.

Changes in plasma-ChE activity in dosed and control animals are shown in Table 20. During the course of the study, plasma-ChE activity levels in dosed and control animals appear to be more stable than RBC-AChE activity. It was reported that plasma-ChE activity was decreased by about 55% in dosed females at wk 7 and by 37.5% in dosed males at wk 3. Mean plasma-ChE activity in

Table 19. RBC-ChE activity in 90-day subchronic rat study using agent GA.[a]

Dose ($\mu g\ kg^{-1}\ d^{-1}$)	Sex	Week of treatment								
		−1	1	%[b]	3	%[b]	7	%[b]	13	%[b]
0	F	4604 (1451)	2949 (370)	64	5472 (801)	119	4483 (441)	97	4457 (261)	97
28.13	F	5272 (589)	3181 (331)	60	4360 (179)	83	3723 (397)	71	4386 (414)	83
56.25	F	5168 (623)	2975 (180)	58	4315 (514)	83	3169 (381)[c]	61	4205 (545)	81
112.50	F	5073 (428)	2421 (184)	48	3428 (174)[c]	68	3404 (364)	67	4229 (721)	83
0	M	3939 (434)	3588 (297)	91	4427 (422)	112	5582 (455)	142	4789 (290)	122
28.13	M	4115 (356)	2549 (311)[c]	62	4106 (557)	99	5025 (98)	>100	4058 (465)	99
56.25	M	4369 (499)	2409 (788)[c]	55	3630 (417)	83	5200 (340)	>100	4601 (553)	>100
112.50	M	3760 (541)	2367 (534)[c]	63	3612 (582)	96	5145 (281)	>100	4126 (491)	>100

[a]Results given in IU/L, mean and (SEM).

[b]Percent of baseline.

[c]$p \leq .05$, different from control value (0 µg/kg).

Source: Bucci et al. (1992a).

Table 20. Plasma-ChE activity in 90-day subchronic rat study using agent GA.[a]

Dose ($\mu g\ kg^{-1}\ d^{-1}$)	Sex	Week of treatment								
		−1	1	%[b]	3	%[b]	7	%[b]	13	%[b]
0	F	1743 (273)	2033 (284)	116	2204 (303)	126	2544 (334)	146	2891 (425)	166
28.13	F	1369 (111)	1416 (125)	103	1540 (138)	112	1794 (188)	131	2311 (245)	169
56.25	F	1233 (135)[c]	1288 (164)[c]	104	1424 (186)[c]	115	1548 (194)[c]	125	1928 (297)	156
112.50	F	1449 (122)[c]	1202 (113)[c]	82	1136 (133)[c]	78	1148 (121)[c]	79	1755 (190)	121
0	M	401 (25)	397 (25)	99	413 (37)	103	383 (23)	96	344 (26)	86
28.13	M	426 (28)	394 (25)	92	361 (22)	85	362 (17)	85	375 (21)	88
56.25	M	415 (13)	351 (13)	85	319 (12)[d]	77	323 (14)[d]	78	330 (18)	79
112.50	M	405 (31)	311 (16)[d]	77	258 (11)[c,d]	64	268 (13)[c,d]	66	360 (32)	89

[a]Results given in IU/L, mean and (SEM).

[b]Percent of baseline.

[c]$p \leq .05$, different from control value (0 µg/kg).

[d]$p \leq .05$, lower than control and preexposure values, based on ANOVA and Dunnett's comparison (ORNL).

Source: Bucci et al. (1992a).

the female controls exhibited a slow increase during the 13-wk test period (from 1743 IU/L at wk −1 to 2891 IU/L at wk 13). A similar response was seen in the two lowest dose groups of females. In males, mean plasma-ChE activity in controls was lower than preexposure levels (401 IU/L at wk −1) at all weeks except wk 3 (413 IU/L). In the dosed groups of males, mean plasma-ChE levels were lower than preexposure values at all sampling times. Statistical analysis of the plasma-ChE activity indicated that mean values were significantly lower than controls in the mid- and high-dose females at wk -1, 1, 3, and 7, but not at wk 13, and in the high-dose males at wk 3 and 7. The plasma-ChE data were reanalyzed by ORNL with ANOVA and Dunnett's and Scheffe's comparisons. This analysis indicated that plasma-ChE levels in males were significantly lower ($p < .05$) than preexposure values at wk 1, 3, 7, and 13 in the mid-dose group and at wk 1, 3, and 7 in the high-dose group. The two highest dose groups were also significantly lower ($p < .05$) than control values at wk 3 and 7 (and at wk 1 for the high-dose group). In females, plasma-ChE levels were not significantly lower than preexposure values, but were significantly lower ($p < .05$) than controls at wk 1, 3, and 7 for all dose groups and at wk 13 for the two highest dose groups.

Dulaney et al. (1985) evaluated the effects of GA on the growth rates of rats given daily subcutaneous doses of 100 μg/kg for 85 d. The dosed animals exhibited reduced growth rates (42% of controls in the first 15 d, 82% of controls in the next 22 d, and 95% of controls from the 38th day to the end of the study). AChE activity was determined in the striatum and the remainder of the brain 24 hr after the last exposure. Mean brain striatal AChE activity was only 13% of the control value; in the remaining parts of the brain the AChE activity was 22% of the control. In cumulative mortality studies, rats (8–11/dose group) were dosed with 75 or 100 μg kg^{-1} d^{-1} for 25 d. In the low-dose group, 1 of 8 animals died on d 10; in the high-dose group, 1 animal died on d 15 and another on d 20. Dosing was continued for an additional 60 d without any further mortality.

3. Chronic Toxicity. Data on the toxicity of GA to humans or animals following long-term exposures were not found in the available literature.

4. Neurotoxicity. There has been concern that organophosphate compounds like GA may have direct toxic effects on the nervous system. Some organophosphate compounds cause a neurotoxic effect (organophosphate-induced delayed neuropathy, OPIDN) that is not associated with AChE inhibition but rather with inhibition and aging of NTE (neuropathy target esterase). Agent GA has not been shown to produce OPIDN in humans. *In vitro* and *in vivo* animal studies have shown that supralethal doses of GA (12 mg/kg) inhibit NTE in antidote-protected chickens (Johnson et al. 1988; Vranken et al. 1982); however, Henderson et al. (1989, 1992) reported no signs of OPIDN in chickens receiving one dose of 0.125 mg/kg by intramuscular (i.m.) injection or repeated injections of 0.07 mg/kg, 5 d/wk, for 90 d. In range-finding studies the intramuscular LD_{50} value was 0.113 mg/kg. Although mild signs of OPIDN were observed in 1 of

2 surviving chickens injected i.m. with 6 mg GA/kg on 2 consecutive d (total dose, 120 fold the LD_{50}) (Willems et al. 1984), these effects were not duplicated in later studies in which chickens were dosed once or twice with 12 mg GA/kg (Johnson et al. 1988). In studies conducted by Bucci et al. (1992a), intraperitoneal injections of GA in CD rats at dose levels to 112.5 μg kg^{-1} d^{-1}, 5 d/wk, for 13 wk did not result in a significant change in brain NTE. Furthermore, there was no clinical evidence of neuropathology, even though blood ChE activity decreased significantly in the dosed animals. Overall, the data suggest that it is unlikely that OPIDN would occur in humans at less-than-lethal doses.

5. Developmental and Reproductive Effects. There are no data evaluating the potential developmental and reproductive toxicity of GA in humans. Limited animal data indicate that such effects are unlikely. In studies in which CD rats were injected intraperitoneally with 0, 75, 150, or 300 μg GA kg^{-1} d^{-1} on gestation d 6–15, no fetal malformations or developmental effects (with the exception of increased preimplantation losses relative to controls) were observed (Bucci et al. 1993a). Because dosing began on gestation d 6, which was near the end or after the time of implantation, the observed preimplantation losses were not considered to be agent related (Bucci et al. 1993a). Blood ChE activity was not monitored during the study. Signs of maternal toxicity (salivation and lacrimation) were seen at all dose levels, and the highest dose produced tremors in some animals. Mean maternal weight gain was also reduced in the high-dose animals when compared to that of controls. Maternal mortality rates were 1/31, 2/32, and 12/33 in the low-, mid-, and high-dose groups, and all deaths were considered to be agent related. Therefore, the lowest dose of 75 μg kg^{-1} d^{-1} can be considered a LOAEL for maternal effects in rats under the conditions of the study.

In the tests in which agent GA (28.1, 56.3, and 112.5 μg kg^{-1} d^{-1}) was administered subcutaneously to New Zealand white rabbits on gestation d 6–19, no adverse effects on fetal implantations, fetal weight, and fetal malformations were observed (Bucci et al. 1993a). However, maternal toxicity (indicated by salivation, diarrhea, and nasal discharge) was evident in the high-dose group, which also experienced a mortality rate of 13.3% (4/30). Therefore, the highest dose of 112.5 μg kg^{-1} d^{-1} can be considered a LOAEL for maternal effects in rabbits.

6. Carcinogenicity. No information is available regarding the potential carcinogenicity of GA in humans. No long-term animal carcinogenicity studies have been carried out on GA. Neoplastic lesions were not observed in male and female CD rats injected intraperitoneally with 28.13, 56.25, or 112.5 μg GA kg^{-1} d^{-1} for 90 d (Bucci et al. 1992a); however, this subchronic study was of insufficient duration to fully evaluate tumor incidence rates. No other animal data are available to assess the potential carcinogenicity of GA.

7. Genotoxicity. No information is available regarding the genotoxicity of GA in humans; however, genotoxicity and mutagenicity data are available from microbial assays and from *in vitro* and *in vivo* tests on laboratory animals (Wilson et al. 1994). GA was found to be weakly mutagenic in 8 of 11 Ames *Salmonella* assays using the revertant strains TA98, TA100, TA1535, and TA1538 and S-9 activation. GA also induced dose-related increases in mutation rates when tested on mouse L5178Y lymphoma cells without metabolic activation; the increase observed at a test concentration of 100 μg/mL was nearly threefold that of the control. An increase in sister chromatid exchanges (SCE) was observed in Chinese hamster ovary cells exposed *in vitro* to GA concentrations of 25–200 μg/mL. Dose responses were linear and highly statistically significant; however, the number of SCEs did not exceed twice the control value at any of the concentrations tested. C57B1/6 mice treated *in vivo* with a maximally tolerated intraperitoneal dose of 700 μg GAB/kg did not exhibit a significant increase in SCE in splenic lymphocytes. Exposure of rat hepatocytes to GA concentrations as high as 200 μg/mL resulted in inhibition of unscheduled DNA synthesis. From the results of these studies (i.e., three positive responses in five assays), Wilson et al. (1994) concluded that GA was a weakly acting mutagen.

B. Estimated Reference Dose

1. Selection of the Key Study. Human data are not available for deriving an RfD_e for agent GA, and the only useful long-term animal data are those for nonoral exposure routes. In one subchronic exposure study, rats were injected intraperitoneally with GA once per day for 90 d (Bucci et al. 1992a), and in another study rats were injected subcutaneously with GA for 85 d (Dulaney et al. 1985). Nonoral exposure data are not normally used for deriving an oral RfD because of difficulties in estimating equivalent dose levels for oral exposures. However, USEPA has used nonoral exposure data to derive an oral RfD for silver, but only because adequate gastrointestinal absorption data were available to estimate equivalent oral doses from the experimental intravenous data (USEPA 1991b). In the absence of an oral dosing study on GA, an RfD_e is derived from the study conducted by Bucci et al. (1992a) with an adjustment for the nonoral exposure route. The Bucci et al. (1992a) study was chosen over the Dulaney et al. (1985) study because Bucci et al. used a series of doses and included a more comprehensive evaluation of the potential toxicity. Ideally, information on the relative absorption of GA through both the oral and intraperitoneal routes should be used to estimate an equivalent oral dose from intraperitoneal data. Because such data are not available, a very provisional estimate of the equivalent oral dose is derived here by comparing the oral and intraperitoneal LD_{50} values for the rat. The reported rat oral LD_{50} value of 3700 μg/kg is 4.6 and 7.6 times larger than the reported intraperitoneal LD_{50}s of 800 and 490 μg/kg, respectively. The assumption is made that a similar relationship would occur under a subchronic exposure protocol.

The use of a rat study for developing an RfD_c for GA is complicated by the fact that rodents have a much lower RBC-AChE activity level compared to humans (Ellin 1981). By itself, this could cause rats to be relatively more sensitive than humans to anticholinesterase compounds; however, the lower RBC-AChE activity may be offset by the presence of aliesterases in the blood of rats. Aliesterases, which are not found in human blood plasma, are known to bind to and therefore reduce the toxicity of GB; a similar mechanism may operate in the case of GA. Other species differences, such as in the rates of aging of the GA-ChE complex, in the rates of synthesis of plasma-ChE in the liver, and in the levels of AChE in the nervous system (Ivanov et al. 1993), may also result in difference between species in sensitivity to GA. Data are insufficient to more fully evaluate these possibilities. There are few human acute toxicity data that can be compared with the available rat data; however, acute toxicity data for primates in general (see Table 18) suggests that humans are likely to be more sensitive than rats. Therefore, for the purpose of this assessment, the standard EPA method, which assumes that humans can be as much as ten times more sensitive to a chemical than laboratory animals, is followed.

2. *RfD_c Derivation.* In the Bucci et al. (1992a) study, 12 CD rats per sex/dose group were injected intraperitoneally with GA at dose levels equivalent to 0, 28.13, 56.25, or 112.5 μg/kg. The injections were given once per day, 5 d/wk, for 13 wk. Details of the study are given in Section X.A.2, Subchronic Toxicity. The only significant changes observed in the dosed animals were decreases in blood ChE activity levels. In the case of RBC-AChE levels, considerable fluctuations occurred between and within test and control groups (0 $\mu g\ kg^{-1}\ d^{-1}$) (see Table 19). Because of the variability in the RBC-AChE data in the control groups, a LOAEL or NOAEL could not be clearly established for the treated groups.

Of the parameters measured in the Bucci et al. study, changes in plasma-ChE values in male rats provided the least variable indication of a LOAEL and NOAEL for GA. Based on the ORNL reanalysis of the data, plasma-ChE was significantly lower ($p < .05$) than both preexposure and control levels at wk 3 and 7 in the two highest dose groups, and significantly ($p < .05$) lower than preexposure and control values at wk 1 in the high-dose group (see Table 20). There is also evidence (based on mean plasma ChE values) of a dose–response relationship for all sampling times (i.e., plasma-ChE was lower at the higher doses). Maximum depression of plasma-ChE occurred at 3–7 wk, a condition also seen in a study of rats dosed with the nerve agent VX (Goldman et al. 1988). Therefore, because of the significantly lower levels of plasma-ChE in male rats (relative to both controls and preexposure vales), the middose of 56.25 $\mu g\ kg^{-1}\ d^{-1}$ is considered a LOAEL for plasma-ChE inhibition. Because of the lack of consistent change in plasma ChE (relative to controls or preexposure values), the dose of 28.13 $\mu g\ kg^{-1}\ d^{-1}$ is considered a NOAEL.

The equivalent oral NOAEL is estimated by comparing oral and intraperitoneal LD_{50} values for the rat and assuming that about the same ratio would apply

for longer term exposures. A rat oral LD_{50} of 3700 μg/kg and intraperitoneal LD_{50} values of 490 and 800 μg/kg (average, 645 μg/kg) have been reported. Therefore, the equivalent oral NOAEL is

$$\text{oral NOAEL} = 28.13 \times \left[\frac{3700}{645}\right] = 161.4 \text{ μg/kg/d}$$

The estimated oral NOAEL of 161 μg kg^{-1} d^{-1} can be used to estimate a human oral reference dose (RfD_o) by first adjusting the NOAEL for a 7 d/wk exposure period by using a factor of 5/7; i.e., 5/7 × 161.4 μg/kg = 115 μg/kg, and then applying the result to the following USEPA formula:

$$RfD_o = \frac{115 \text{ μg/kg/d}}{UF_H \times UF_A \times UF_S \times UF_L \times UF_D \times MF}$$

where:

UF_H = 10 (sensitive subpopulations)
UF_A = 10 (animal to human extrapolation)
UF_S = 3 (extrapolation from subchronic to chronic exposures).
UF_L = 1 (LOAEL to NOAEL extrapolation)
UF_D = 3 (data base incomplete)
MF = 3 (modifying factor)

A total uncertainty factor of 3000 was applied, allowing for protection of sensitive subpopulations (10), subchronic-to-chronic extrapolation (3), animal-to-human extrapolation (10), the lack of a complete data base (3), and the inclusion of a Modifying Factor (3) because the RfD_o was based on a nonoral study. A UF_H of 10 for sensitive subpopulations is considered necessary because some individuals have abnormally low levels of blood ChE activity, which may make them especially susceptible to the effects of ChE inhibitors such as nerve agents (see Section III.B.6 for additional discussion). The standard uncertainty factor of 10 is used for animal-to-human extrapolation because there is no evidence to suggest that humans are less sensitive to GA than rodents.

A UF_S of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of oral RfDs for other organophosphate compounds, EPA has used NOAELs for ChE inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a UF_S of 1 was used). In addition, animal data for other organophosphate ChE inhibitors such as agent VX indicate that maximum ChE inhibition usually occurs 30–60 d after exposure begins and then levels off or even shows signs of recovery. However, an uncertainty factor of 3 is used here because chronic studies are not available to verify that additional effects would not occur following chronic exposures.

The data base for GA consists of two subchronic toxicity studies in rats, teratology studies in two species (rats and rabbits), and delayed neuropathy studies in chickens. These studies generally support the use of ChE inhibition as the

critical endpoint for deriving an oral RfD_e. Deficiencies in the data base include the lack of a multigeneration reproductive toxicity study, a standard toxicity study in a second species, and toxicity studies by the oral exposure route. Because studies on other organophosphate ChE inhibitors, including a multigenerational study on agent VX, indicate that reproductive effects are unlikely, a full UF_D of 10 is not considered necessary for data base deficiencies.

The key study involved a nonoral exposure route (intraperitoneal) and required route-to-route extrapolation using acute toxicity data. Because of uncertainties associated with the use of this nonstandard methodology, a Modifying Factor of 3 was applied to the RfD_e.

Therefore:

$$RfD_e = \frac{115\ \mu g/kg/d}{10 \times 10 \times 3 \times 1 \times 3 \times\ 3}$$

$$RfD_e = 0.04\ \mu g\ GA/kg/d$$

3. Overall Confidence in the Oral RfD_e.

Study: Medium
Data base: Low
RfD_e: Low

The data base for GA consists of intraperitoneal and subcutaneous subchronic studies in rats, teratology studies in rats and rabbits, and delayed neuropathy studies in rats and chickens. Deficiencies in the data base include the lack of a multigeneration reproductive toxicity study, a standard toxicity study in a second species, and adequate toxicity studies by the oral exposure route. Although well designed and well conducted, the principal study involved a nonoral (intraperitoneal) exposure route. Consequently, overall confidence in the RfD_e is low.

4. Comparison of the Oral RfD_e with Human Toxicity Data. There are no human data available for GA for the oral exposure route. The intravenous LD_{50} was estimated to be 0.014 mg/kg (Robinson 1967). Inhalation data indicate that severe effects would occur at a concentration of 50 mg-min/m^3 (Reutter and Wade 1994). The LCt_{50} is 135 mg-min/m^3 for time periods of 0.5–2.0 min (DA 1974). DHHS (1988) has set an inhalation control limit of 0.000003 mg/m^3 for the general public (72-hr time-weighted average).

XI. Agent GB (Sarin)

Agent GB (sarin) is an organophosphate ChE inhibitor similar in mode of action and toxic effects to agents GA, GD, and VX. Agent GB is volatile and also soluble in water. It is acutely toxic by all routes of exposure, inhalation, inges-

tion, and ocular and percutaneous absorption (DA 1974). It is not as toxic as VX when administered percutaneously, comparable in toxicity to GD, but more toxic than GA. The toxicity of nerve agents has been reviewed in several earlier reports (Carnes and Watson 1989; Dacre 1984; Munro et al. 1994; Sidell 1992; Somani et al. 1992; Watson et al. 1989b). This review focuses primarily on the available subchronic or chronic oral toxicity data that may be useful for deriving an oral RfD_o. The reader is referred to the above-cited references for additional information on the acute toxicity of agent GB.

A. Toxicology

1. Acute Toxicity.

Human Studies. In tests on humans, Grob and Harvey (1958) found that a single oral dose of 0.022 mg GB/kg produced mild toxic effects including anorexia, nausea, heartburn, tightness in the stomach and chest, increased fatigue, nervous tension, anxiety, and other CNS responses including insomnia and excessive dreaming. An additional dose of 0.008 mg/kg within 8 hr resulted in moderate toxic effects including stomach cramps, vomiting, diarrhea, increased salivation and lacrimation, slightly decreased heart rate, and abnormal breathing. According to Thienes and Haley (1972), a single dose of 0.002 mg GB/kg caused excessive dreaming and talking during sleep, and a dose of 0.020 mg/kg caused insomnia, excessive dreaming, withdrawal, and depression. At high exposures, brain damage may occur as a result of oxygen deprivation in brain tissue during GB-induced convulsions (Sidell 1992).

Grob and Harvey (1958) reported that the first appearance of toxicity in humans occurred when RBC-ChE activity was depressed 88% (to 12% of the baseline value) following a single oral dose of GB. The single-dose oral ChE_{50} value was reported to be 0.01 mg GB/kg, and the lethal oral dose was estimated to be 0.14 mg/kg. In comparison, the single-dose intraarterial ChE_{50} was reported to be 0.003 mg/kg and the lethal intramuscular dose was estimated to be 0.03 mg/kg. Following intravenous (i.v.) administration, toxic effects occurred when RBC-AChE activity was depressed 40%–50% (60%–50% of baseline), indicating a more immediate effect on the nervous system than that caused by oral dosing (Grob and Harvey 1958). In inhalation studies conducted on human volunteers, small changes in single-fiber electromyography (SFEMG) were measured in individuals exposed to 0.5 mg GB/m^3 for 30 min (Baker and Sedgwick 1996). RBC-AChE was reduced to 60% of normal in the exposed individuals, some of whom exhibited miosis and mild dyspnoea. The SFEMG returned to normal 2 yr after exposure.

Grob and Harvey (1958) administered multiple oral doses of GB to human volunteers during a 3-d period (3–24 hr apart; average, 7.5 hr). In two individuals, doses of 0.0005 or 0.005 mg/kg, totaling 0.007 mg/kg over the 3-d period, reduced RBC-ChE 33% and 27%, respectively, but neither produced toxic effects. Multiple doses of 0.008–0.016 mg/kg, totaling 0.088 mg/kg over the 3-d

period, produced mild symptoms of toxicity. Similar incremental doses, totaling 0.102 mg/kg during 3 d, produced moderate symptoms of toxicity and greater than 90% reduction in RBC-ChE activity. Grob and Harvey (1958) reported that exposure to GB had a cumulative effect that resulted in increased sensitivity to the chemical. Human LD_{50} values estimated from animal data are shown in Table 21.

Animal Data. LD_{50} data for selected species are shown in Table 21. A concentration of 4.34 ppm GB in the drinking water of rats (equivalent to about 0.1 mg GB kg^{-1} d^{-1}) for 9 d resulted in signs of toxicity (partial paralysis of the hind legs) within the first 1–4 d of the exposure period (Bauer et al. 1949). In range-finding studies conducted with GB type I (GB containing tributylamine as a stabilizer) and GB type II (GB containing diisopropylcarbodiimide as a

Table 21. Lethality data for agent GB.

Exposure route	Species[a]	LD_{50} (μg/kg)	References
Oral	Human	71–285[b]	Somani et al. (1992)
	Human	140[c]	Grob and Harvey (1958)
	Rat	550	RTECS (1995c)
	Rat	600	Grob and Harvey (1958)
	Rat	870–1060	Bauer et al. (1949)
Dermal	Human	28,000	RTECS (1995c)
	Human	24,000	DA (1974)
	Human	1,429–7,143[b]	Somani et al. (1992)
	Pig	115,900	DA (1974)
	Rat	2500	DA (1974)
	Mouse	1080	RTECS (1995c)
Intravenous	Human	14	DA (1974)
	Monkey	20	DA (1974)
	Monkey	15	Woodard et al. (1994)
	Pig	15	DA (1974)
	Rat	39	Fleisher et al. (1963)
		45	DA (1974)
Subcutaneous	Rat	103–158	RTECS (1995c) Harris et al. (1984)
Intramuscular	Human	30[c]	Grob and Harvey (1958)
	Monkey	22	RTECS (1995c)
	Rat	170	Grob and Harvey (1958)
		108	RTECS (1995c)
		112	DA (1974)
Intraperitoneal	Rat	218	Fleisher et al. (1963)
		250	RTECS (1995c)

[a]Values for humans estimated from animal data.
[b]Based on 70-kg body weight.
[c]Lethal level.

stabilizer), Bucci et al. (1991) and Bucci and Parker (1992) found that a dose of 0.5 mg GB/kg, when administered by gavage once per day, 5 d/wk, for 3 wk, was lethal to rats; the maximum tolerated dose was 0.3 mg kg^{-1} d^{-1}. Doses of GB sufficiently high to cause convulsions also cause brain lesions and cardiomyopathy in rats (Singer et al. 1987; see also McLeod 1985 for review).

2. *Subchronic Toxicity.* In subchronic toxicity studies conducted by the National Center for Toxicological Research (NCTR), male and female CD rats (12 per sex/dose group) were administered GB type I (GB containing tributylamine as a stabilizer) or GB type II (GB containing diisopropylcarbodiimide as a stabilizer) by gavage at dose levels equivalent to 0, 0.075, 0.15, or 0.3 mg GB/kg (Bucci and Parker 1992; Bucci et al. 1991). The agent was administered once per day, 5 d/wk, for 13 wk. The test animals were observed daily for clinical signs of toxicity and weighed weekly. Necropsy examination was performed on all animals and terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals and on those tissues of lower-dose animals that were abnormal at necropsy. Hematological analyses and clinical chemistry (including RBC- and plasma-ChE) were evaluated in the same 6 male and 6 female rats in each dose group 1 wk before the exposures began and also at wk 1, 3, 7, and 13. In addition, at necropsy a hemisection of each brain was prepared and tested for NTE activity.

In both studies there were several statistically significant changes in clinical chemistry (i.e., aspartate aminotransferase in mid-dose males exposed to GB type II) and hematology (decrease at wk 7 in white blood cells in high-dose females exposed to GB type II and increase at wk 13 in erythrocytes in mid-dose females exposed to GB type II); however, these effects were not sufficiently consistent to suggest organ dysfunction. Brain NTE was not altered significantly in any rats dosed with GB type II; however, it was significantly decreased ($p < .05$) in female rats dosed with 0.3 mg kg^{-1} d^{-1} GB type I. The latter, however, did not exhibit any histological signs of delayed neuropathy. GB type II was not associated with any neoplastic or nonneoplastic lesions. Two high-dose females and one low-dose female dosed with GB type I had brain lesions consisting of necrosis and vacuolization of individual hippocampal pyramidal cells. It was reported by Bucci et al. (1991) that this type of lesion is consistent with hippocampal hypoxia resulting from the respiratory convulsant effects of GB; however, Bucci et al. (1991) also noted that postmortem autolysis could have mimicked cerebral necrosis in two of the animals that were found dead. The third animal exhibited signs consistent with nerve agent toxicity (e.g., rapid breathing, salivation, lacrimation, hemorrhage in the urinary wall, and possible right forelimb paralysis), and the observed neural lesions were attributed to GB. This animal was in the test group receiving 0.075 mg kg^{-1} d^{-1}. As noted, none of the rats dosed with up to 0.3 mg kg^{-1} d^{-1} of GB type II exhibited brain lesions (GB type I contains the stabilizer tributylamine and GB type II contains diisopropylcarbodiimide). Subchronic and chronic toxicity data for these stabi-

lizers are lacking and, in terms of acute toxicity, the stabilizers have only a fraction of the toxicity of the nerve agents (e.g., the oral LD_{50} for tributylamine is 114 mg/kg in rats) (RTECS 1995b). Although there is one report indicating that tributylamine is a CNS stimulant (Windholz et al. 1983), there is no evidence to suggest that it contributed to the neurotoxic effect seen in the GB type I study.

In the NCTR studies, RBC-AChE activity in the dosed animals was compared to control values for the same sampling times (Tables 22 and 23). In both studies significant decreases in plasma and RBC-ChE activity were seen at certain time periods; however, the results for the GB type II study (Table 22) were more internally consistent than those for GB type I (Table 23); i.e., the control values did not vary as greatly, and the test groups in general exhibited a more clearly defined dose response. Inhibition of RBC-AChE by GB type II was dose related for females in the two highest dose groups and for males in all dose groups (Table 22). Maximum RBC-ChE depression (48%) occurred in wk 7 in both high-dose males and females. Male rats exposed to the lowest dose of GB type II exhibited a 38% decrease in RBC-ChE activity in wk 1; females exhibited a 12% decrease. By wk 13, RBC-ChE activity levels in females returned to near preexposure levels (>90%); however, levels in males were still depressed 16%–24%. When the AChE data for GB type II were reanalyzed by ORNL with ANOVA and Dunnett's and Scheffe's comparisons, RBC-AChE activity in males was significantly lower ($p < .05$) than baseline and control values in all dose groups at wk 1, 3, and 7. Similar results were seen in females except that RBC-AChE activity was not significantly different from controls or baseline values in the low-dose group. Effects on plasma ChE activity were not as great as those on RBC-AChE; significant inhibition of plasma ChE occurred primarily in the two high-dose groups (Table 24).

In a subchronic inhalation study conducted on Fischer-344 rats, no signs of toxicity were observed in animals exposed to 0.0001 or 0.001 mg GB/m^3, 6 hr/d, 5 d/wk for as long as 24 wk (Weimer et al. 1979). Compared to the dose levels used in the subchronic oral studies described previously, the exposures used in the Weimer et al. (1979) study were relatively low. For example, assuming an inhalation rate of 0.29 m^3/d and an average body weight of 0.35 kg for rats and 100% pulmonary absorption, the highest concentration in the Weimer et al. study would be equivalent to an internal dose of only 0.00015 mg kg^{-1} d^{-1}. In another subchronic inhalation study, rats were exposed to a daily Ct of 50 mg-min/m^3 (1 mg/m^3 for 50 min), 5 d/wk, for 88 d (Cohen et al. 1954). During the first few days immediately after the exposure, and while RBC-ChE activity was more than 50% of the normal controls and brain ChE was not significantly different from control values, some of the exposed animals exhibited hypertonicity and hyperactivity of musculature with increased response to stimuli, rigidity, and convulsions. Using the same parameters as listed earlier, the equivalent internal dose in this study is estimated to have been about 0.03 mg kg^{-1} d^{-1}, only about one-tenth of the highest dose used in NCTR studies. This comparison

Table 22. RBC-ChE activity in 90-day subchronic study of GB type II in CD rats.[a]

Dose ($\mu g\ kg^{-1}\ d^{-1}$)	Sex	Week of treatment							
		−1	1	%[b]	3	%[b]	7	%[b]	13	%[b]
0	F	1728 (174)	1777 (223)	103	1497 (182)	87	1828 (280)	106	2313 (285)	130
75	F	1603 (149)	1405 (67)	88	1545 (221)	96	1353 (135)	84	1753 (249)	109
150	F	1678 (132)	1060 (54)[c,d]	63	1085 (62)	65	985 (83)[c,d]	59	1553 (161)[c]	93
300	F	1653 (75)	968 (41)[c,d]	59	895 (41)[d]	54	865 (87)[c,d]	52	1510 (79)[c]	91
0	M	1118 (20)	1032 (40)	92	1270 (42)	114	1002 (76)	90	1097 (54)	98
75	M	1180 (78)	728 (33)[d]	62	910 (47)[c,d]	77	748 (96)[c,d]	63	992 (72)	84
150	M	1097 (46)	665 (51)[d]	61	782 (73)[c,d]	71	602 (36)[c,d]	55	835 (78)[c]	76
300	M	1156 (59)	612 (25)[d]	53	764 (91)[c,d]	66	606 (30)[c,d]	52	910 (97)[c]	79

[a]Mean IU/L and (SEM).

[b]Percent of baseline (wk −1).

[c]$p < .05$, different from control value (0 µg/kg).

[d]$p \leq .05$, lower than control and preexposure values, based on ANOVA and Dunnett's comparison (ORNL).

Source: Bucci and Parker (1992).

Table 23. RBC-ChE activity in 90-day subchronic study of GB type I in CD rats.[a]

Dose (µg/kg)	Sex	Week of Treatment							
		−1	1	%[b]	3	%[b]	7	%[b]	13	%[b]
0	F	2343 (118)	1885 (196)	80	1765 (143)	75	3177 (118)	136	1533 (375)	65
75	F	2420 (168)	1964 (121)	81	1584 (109)	65	2101 (94)[c]	87	1265 (383)	52
150	F	2656 (94)	1814 (188)	68	1867 (143)	70	2217 (158)[c]	83	1318 (288)	50
300	F	2518 (127)	1564 (99)	62	1557 (97)	62	2061 (126)[c]	82	1673 (441)	66
0	M	2085 (267)	1771 (205)	85	1954 (97)	94	2346 (124)	113	1547 (285)	74
75	M	2219 (114)	1886 (282)	85	1834 (53)	83	1749 (82)	79	1405 (166)	63
150	M	2444 (177)	1835 (151)	75	1907 (104)	78	1917 (135)	78	1522 (173)	62
300	M	2632 (218)	1921 (126)	73	1990 (96)	76	2006 (94)	76	1478 (98)	56

[a]Values given as mean IU/L and (SEM).
[b]Percent of baseline.
[c]$p < .05$, different from control value (0 µg/kg).
Source: Bucci et al. (1991).

Table 24. Plasma ChE activity in 90-day subchronic study of GB type II in CD rats.[a]

Dose (µg/kg)	Sex	Week of treatment								
		−1	1	%[b]	3	%[b]	7	%[b]	13	%[b]
0	F	1267 (189)	1935 (304)	153	1461 (246)	115	1761 (353)	139	2100 (350)	166
75	F	1381 (288)	1460 (206)	106	1483 (350)	107	1475 (188)	107	1574 (280)	114
150	F	1136 (105)	705 (104)[c,d]	62	736 (53)[d]	65	790 (136)[c]	70	1280 (149)	113
300	F	1316 (55)	611 (73)[c,d]	46	475 (58)[c,d]	36	481 (95)[c,d]	36	1311 (146)	100
0	M	437 (33)	578 (59)	132	353 (29)	81	254 (15)	58	313 (18)	72
75	M	461 (25)	407 (53)	88	296 (22)	64	174 (12)[d]	38	312 (25)	68
150	M	443 (40)	239 (26)[c,d]	54	229 (9)[d]	52	122 (7)[d]	28	246 (13)[d]	56
300	M	375 (19)	249 (52)[c,d]	66	187 (15)[d]	50	109 (9)[d]	29	257 (4)[d]	69

[a]Mean IU/L and (SEM).

[b]Percent of baseline (wk −1).

[c]$p < .05$, different from control value (0 µg/kg).

[d]$p \leq .05$, lower than control and preexposure values, based on ANOVA and Dunnett's comparison (ORNL).

Source: Bucci and Parker (1992).

would suggest that the oral exposure pathway is much less toxic than the inhalation pathway, possibly as a result of hydrolysis of the agent in the gastrointestinal tract.

Dulaney et al. (1985) evaluated the effects of GB on mortality and growth rates of rats given daily subcutaneous injections of 25, 50, 65, or 75 μg GB/kg. None of the 5 animals dosed with 25 μg kg^{-1} d^{-1} for 85 d died, but growth rates were reduced (80% of controls in the first 20 d, 70% of controls in the next 7 d, and 89% of controls from the 29th day to the end of the study). One of the 5 animals dosed with 50 μg kg^{-1} d^{-1} for 85 d died and growth rates were lower than those in the low-dose group (71% of controls in the first 35 d and 85% of controls thereafter). AChE activity was determined in the striatum and the remainder of the brain 24 hr after the last exposure. Mean brain striatal AChE activity was only 90% and 66% of the control values for the low- and high-dose groups, respectively; in the remaining parts of the brain, the AChE activity was near control values in the low-dose group and 64% of the control in the high-dose group. Seven of 11 animals dosed with 65 μg kg^{-1} d^{-1} died during a 25-d exposure period, and 6 of 8 dosed with 75 μg kg^{-1} d^{-1} died during the same time period.

3. Chronic Toxicity.

Human Studies. No information is available on the effects of agent GB in humans following chronic exposures to low concentrations. However, in a retrospective electroencephalogram (EEG) study of workers who had had at least one episode of accidental exposure to agent GB (1 yr or more before testing), Duffy et al. (1979; see also Burchfiel and Duffy 1982) found that the exposed workers exhibited a significant increase in brain beta activity (12–30 Hz), as well as increased levels of REM (rapid-eye-movement) sleep. In evaluating these studies, DHHS (1987) considered the EEG changes to be "of questionable significance—given the difficulty of demonstrating such changes and the absence of clinically significant effects even when EEG changes are present."

Animal Data. There are no animal studies involving chronic oral exposures to GB. In chronic inhalation studies conducted by Weimer et al. (1979), ICR Swiss and A strain mice, Sprague-Dawley/Wistar and Fischer-344 rats, and purebred beagle dogs were exposed to 0, 0.0001, or 0.001 mg GB/m^3, 6 hr/d, 5 d/wk for up to 52 wk. Four male and 8 female beagles were exposed to each test concentration. In the rodent studies, 50 animals of each sex of each strain were exposed to each test concentration. The control groups were identical to the test groups except that an additional 100 F344 rats and A strain mice were used. Animals were killed according to the schedule listed in Table 25. RBC-AChE activity levels were monitored throughout the study for all test species. No dose-related, statistically significant changes in RBC-AChE occurred in any species at any sampling time. Using an inhalation rate of 0.34 m^3/d for rats (USEPA 1994a), and assuming 100% pulmonary absorption, the 6 hr/d, 5 d/wk exposure would

Table 25. Sacrifice schedule for agent GB chronic inhalation study.

	Number of animals killed								
	Months of exposure								Post exposure
Species	1	2	3	4	5	6	9	12	(6 mon)
Colony rats	10	10	10	10	10	10	10	10	20
Fischer-344 rats	—	—	20	—	—	20	20	20	20
Colony mice	10	10	10	10	10	10	10	10	20
A strain mice	—	—	20	—	—	20	20	20	20
Beagle dogs	2	2	2	—	—	2	2	2	—

Source: Weimer et al. (1979).

correspond to an average daily dose of about 0.0001 mg/kg. This dose is considerably below the gavage doses of 0.075 mg kg^{-1} d^{-1} that produced ChE depression in the subchronic studies described previously.

Five of 20 dogs exhibited abnormal EKGs at the time of sacrifice. Elevated P-waves were suggestive of right atrial hypertrophy; however, there was no evidence of enlargement or physical abnormalities of the heart on autopsy. The absence of preexposure data precludes identifying this effect as caused by GB exposure. A higher incidence of tracheitis occurred in colony rats (a Sprague-Dawley/Wistar population) and in Fischer rats exposed to GB in comparison to control animals (Table 26). The most severe cases occurred in the high-exposure group. The investigators could not determine whether the occurrence of tracheitis was agent related. No other overt signs of GB-related toxicity were observed at either exposure level. Atrophy of the seminiferous tubules, starting at 12 wk

Table 26. Incidence of tracheitis in colony rats exposed to agent GB.

	Exposure group		
Exposure period (wk)	Control	0.0001 mg/m^3	0.001 mg/m^3
4	0/10	5/10	0/10
8	0/10	4/10	9/10
12	0/10	5/8	5/7
16	0/9	0/10	1/10
20	0/10	0/5	2/6
24	1/10	1/5	1/10
36	0/9	2/5	2/7
52	2/20	1/20	6/20
Post exposure, 6 mon	0/20	7/19	9/28
Totals	3/108	25/98	34/108

Source: Weimer et al. (1979).

of exposure, was also seen in the Fischer rats. The investigators noted that this inbred strain of rat is susceptible to numerous genetically based defects that may appear under experimental conditions of stress. The tests were repeated using the same experimental protocol for 12 and 24 wk. None of the rats in this second assay exhibited testicular atrophy (Morin and McKinley 1976).

4. Neurotoxicity. Occupational exposures to agent GB have been associated with subtle neurological changes manifested as altered EEGs (Burchfiel and Duffy 1982; Duffy et al. 1979). Burchfiel and Duffy (1982) evaluated the waking and sleep EEGs of 77 industrial workers, each of whom had had at least one documented episode of exposure to sarin. No exposure, however, had occurred in the year preceding the study. Statistical comparisons were made to a matched but unexposed control group. Spectral analysis of the EEGs indicated significant increases in brain beta activity (12–30 Hz) in the exposed group, and sleep EEGs indicated significantly increased rapid eye movement in the exposed group. Computer analysis of combinations of EEG components was also done in an attempt to identify an exposed individual by EEG characteristics; however, the results were inconclusive. Burchfiel and Duffy (1982) concluded that the EEGs of some of the workers may not have been affected by their exposure to sarin, and that there might be a threshold for this type of effect. Increases in high-frequency beta activity were also observed by Burchfiel and Duffy (1982) in the EEGs of rhesus monkeys who had been injected with sarin 1 yr previously under two different dosing schedules: (1) one 5 μg/kg intraveneous injection or (2) a series of intramuscular injections of 1 μg/kg, given once per week for 10 wk. Control animals did not show any changes in EEG. Burchfiel and Duffy (1982) attributed the changes in EEG activity to the GB exposure; however, they also noted that a clear relationship has not been established between such alterations in EEG frequency spectrum and alterations in brain function. Neurobehavioral tests were not conducted on the exposed animals. In evaluating the data of Burchfiel and Duffy (1982), DHHS (1988) considered the EEG changes to be "of questionable significance—given the difficulty of demonstrating such changes and the absence of clinically significant effects even when EEG changes are present."

Other animal studies have shown that acute and subacute exposure to GB can result in neurobehavioral changes in the test animals. Single intramuscular (i.m.) injections of 6 μg GB/kg to marmosets resulted in adverse behavioral effects when the animals were tested for hand–eye coordination, but no adverse effects were seen in a visual discrimation test (Wolthuis 1992). A dose of 3 μg/kg had no adverse effects on behavior, and hand–eye coordination was improved in three of six animals (Wolthuis 1992). An intraperitoneal (i.p.) dose of 50 μg GB/kg resulted in decreases in rearing and grooming behavior and locomotive activity in male Wistar rats (Nieminen et al. 1990). A subcutaneous (s.c.) injection of 61 μg GB/kg increased spontaneous motor activity in male Sprague-Dawley rats; a dose of 71 μg/kg produced conditioned flavor aversions; 84 and 115 μg/kg caused significant decreases in spontaneous locomotive activity; and

doses of 98 and 115 μg/kg resulted in significant decrements in rotorod performance (Landauer and Romano 1984).

Organophosphate-induced delayed neuropathy (OPIDN) has not been observed in humans exposed to acutely toxic levels of GB [see Munro et al. (1994) for review] nor in cats receiving single supralethal doses or multiple low doses of GB for as long as 10 d (Goldstein 1989; Goldstein et al. 1987). In subchronic rat studies, Bucci et al. (1991) found that male and female rats receiving 0.3 mg GB type I kg^{-1} d^{-1} for 90 d had reduced brain NTE activity levels (significant at the $p < .05$ level in females) but no histopathological signs of OPIDN. Decreases in brain NTE were not seen in a related study in which rats were dosed with the same amount of GB type II (Bucci and Parker 1992). However, signs suggestive of OPIDN have been observed in female Swiss albino mice exposed to 5 mg GB/m^3 for 20 min daily for 10 d (Husain et al. 1993). Muscular weakness of the limbs and slight ataxia occurred on the fourteenth day after the start of the exposures. These changes were accompanied by significant ($p < .001$) inhibition of NTE activity in the brain (59.2%), spinal cord (47.4%), and platelets (55.4%). Histological examination of the spinal cord revealed focal axonal degeneration, which was reported to be moderate in two animals and light in four. The same exposure inhibited AChE in blood by 27.3% and in brain by 19.2% but was not associated with any cholinergic symptoms.

Several studies have also demonstrated that GB can also induce OPIDN in chickens. The single intramuscular dose necessary to produce ataxia in antidote-protected chickens was reported by Davies et al. (1960) to be 650–760 μg/kg, 26–30 times the LD_{50}. Nine of 28 chickens dosed with 1.0 mg GB/kg (given in 20% daily aliquots) exhibited neurotoxic effects, with the mean time for onset of ataxia being 12 d (Davies et al. 1960). Five of 8 chickens injected i.m. with 25 μg GB/kg, once per day for 26–28 d, also exhibited signs of ataxia (Davies and Holland 1972). OPIDN has also been observed in chickens injected s.c. with 30–60 times the GB LD_{50} (Gordon et al. 1983) and in chickens injected s.c. with 50 μg GB/kg (1/10th the LD_{50}), once per day for 10 d (Husain et al. 1995). However, Bucci et al. (1992b) reported that White Leghorn chickens (atropine-protected) given single oral doses of 70.2, 140.4, or 280.7 μg GB type II/kg exhibited no clinical signs of OPIDN when evaluated 8–43 d after the treatment. At death, samples of nervous tissue examined microscopically showed no evidence of pathology. In a related study conducted by Bucci et al. (1992b), hens were administered the same total doses of GB but in one-third increments given 1 wk apart. There were no signs of neuropathology when the animals were killed 43 d later. In a third study, Bucci et al. (1992b) evaluated the effects of GB on NTE in hens given single doses of GB type II ranging from 70.2 to 750 μg/kg. Bucci et al. (1992b) reported that under the conditions of the test, GB type II did not cause a significant dose-related change in NTE in the brain or spinal cord.

5. Developmental and Reproductive Effects. No data are available to evaluate the potential reproductive and developmental effects of GB in humans; however,

studies in laboratory animals indicate that such effects are not likely even at dose levels that are maternally toxic. LaBorde and Bates (1986; see also LaBorde et al. 1996) conducted developmental toxicity studies on agent GB type I and GB type II using CD rats and New Zealand rabbits. In the rat studies, the test animals were dosed with 0, 100, 240, or 380 μg GB/kg orally on d 6–15 of gestation. Females were weighed on gestational d 0, 6–16, and before death on gestational d 20. The test animals were observed for clinical signs of toxicity. At sacrifice, gravid uteri were weighed and examined for number and status of implants (alive, resorbed, or dead). Individual fetal body weight and internal or external malformations were recorded. Maternal toxicity (evidenced by excessive salivation, ataxia, lacrimation) and mortality (8/29 for GB type I and 13/29 for GB type II) occurred in the high-dose group. There were no significant differences among treatment groups in the incidence of resorptions or in the average body weight of live fetuses per litter. The only fetal morphological anomaly was fetal hydroureter, which occurred at a rate of 5.2%, 1.9%, 5.3%, and 2.1% with GB type I and 4%, 5%, 3.2%, and 0.5% with GB type II in the 0, 100, 240, and 300 μg/kg dose groups, respectively. The observed effect was not dose related and was therefore considered to be a spontaneous variant. Skeletal and cartilage variants occurred between dose groups but these were not statistically significant. In similar studies conducted on New Zealand rabbits using the same experimental protocol, oral doses of 0, 5, 10, or 15 μg kg^{-1} d^{-1} on gestational d 6–19, resulted in no fetal toxicity or teratogenicity (Laborde and Bates 1986). The only observed fetal anomaly was retinal folding, which occurred at a rate of 6.8%, 3.9%, 4.3%, and 7.4% for GB type I and 17%, 18%, 25%, and 19% for GB type II in the 0, 5, 10, and 15 μg/kg dose groups, respectively. The frequency of the anomaly was not dose related and the variant was, therefore, considered to be a spontaneously occurring malformation. Maternal toxicity, evidenced by excessive salivation, ataxia, and lacrimation, occurred at the highest dose.

The reproductive and developmental toxicity of GB was also evaluated in a pilot study in which Sprague-Dawley rats were exposed to GB vapors (0.1 or 1 $\mu g/m^3$) or injected i.p. with the agent (Denk 1975). In one series of inhalation tests, male rats were exposed for 6 hr/d, 5 d/wk, for 1, 2, 8, or 12 wk, or 6, 9, or 12 mon, and then mated to unexposed females. Nineteen days after mating, the females were killed and examined for number of corpora lutea, deciduomata, number of fetal deaths, and number of live fetuses. Mated pairs of rats were also exposed to the same GB concentrations for 1, 2, or 3 wk or until the pups were whelped. The incidence of intrauterine deaths was recorded, and all fetuses were examined for abnormalities. In a third series of tests, males and females were exposed to GB for 10 mon and then mated. The F_1 generation was mated at 12 wk of age, as was the F_2 generation. The number and sex of offspring, number of preweaning deaths, number weaned, and pup weights at various ages were recorded. In the intraperitoneal tests, males were given single doses of GB (13.67, 27.34, 54.75, 109.5, or 219.02 μg/kg) and then mated to unexposed females 7 or 14 d after treatment, or mated pairs were injected with GB (43.8

μg/kg) on the same day as mating or after 7, 14, or 21 d. Denk (1975) reported reduced rates of whelping in the F_0 generation in the multigeneration study, but reduced whelping rates were also seen in the controls, and this effect was thought to be caused by the age of the animals at mating (12 mon). No other adverse effects with respect to dominant lethal mutations, reproductive performance, fetal toxicity, and teratogenesis were observed.

6. Carcinogenicity. There are no human data to suggest that GB is carcinogenic. As part of chronic inhalation studies conducted by Weimer et al. (1979) (see Section XI.A.3, Chronic Toxicity), the tissues of animals exposed to GB for as long as 1 yr were examined for microscopic lesions including tumors. The test species included ICR Swiss mice, A strain mice, Sprague-Dawley/Wistar rats, Fischer-344 rats, and purebred beagle dogs; the exposures were to 0.0001 or 0.001 mg GB/m^3, 6 hr/d, 5 d/wk. Weimer et al. (1979) reported that agent-related tumors did not occur in any of the exposed species. Pulmonary tumors did occur in strain A mice; after 52 wk of exposure, pulmonary adenomas were present in 3/19 animals exposed to 0.0001 mg GB/m^3, in 3/20 animals exposed to 0.001 mg GB/m^3, and in 0/20 controls; for animals maintained for 6 mon post exposure, the incidence rates for pulmonary adenocarcinomas were 5/19, 6/18, and 9/29, respectively. However, these lesions were not considered to be agent related. Strain A mice have a high natural propensity to form pulmonary tumors, the incidence of spontaneous pulmonary tumors being about 53% in animals 12 mon of age and 90% in animals 18 mon of age (Heston 1942). Overall, the studies of Weimer et al. (1979) indicated that agent GB is not carcinogenic.

7. Genotoxicity. No information was found in the available literature regarding the genotoxicity of GB in humans. In bioassays using bacteria and mammalian cell cultures, GB was not genotoxic or mutagenic when tested with or without metabolic activation (Goldman et al. 1987). GB did not induce biologically significant increases in mutations when tested in the Ames *Salmonella* assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) (Goldman et al. 1987). GB type I and GB type II did not induce a significant increase in forward mutations when tested on mouse L5178Y lymphoma cells at concentrations of 50, 100, or 200 μg/mL (Goldman et al. 1987). An increase in SCEs was not observed in Chinese hamster ovary cells exposed *in vitro* to 200 μg/mL of GB (Goldman et al. 1987). Mice treated *in vivo* with a maximally tolerated i.p. dose of 360 μg GB/kg did not exhibit a significant increase in SCE in splenic lymphocytes (Goldman et al. 1987). Exposure of rat hepatocytes to GB concentrations as high as 2.4×10^{-3} M resulted in a decrease in DNA repair synthesis, leading Goldman et al. (1987) to conclude that GB probably did not damage DNA directly but that it might inhibit DNA synthesis after non-agent-induced DNA damage had occurred.

B. Estimated Reference Dose

1. Selection of the Key Study. Long-term human exposure studies provide the most useful data on which to base an RfD_c; however, such data are not available for agent GB. The only subchronic or chronic exposure studies conducted on GB consist of a 90-d study in which rats were given GB type I (Bucci et al. 1991) or GB type II (Bucci and Parker 1992) by gavage, and a 1-yr study in which rats, mice, and dogs were exposed to GB by inhalation (Weimer et al. 1979). For the development of an oral RfD_c, a study involving the same exposure pathway is preferred even though the exposure period may be less than chronic. Therefore, the subchronic rat studies are considered to be more relevant than the inhalation studies for deriving an RfD_c for agent GB.

In the Bucci and Parker (1992) study conducted with GB type II, statistically significant ($p < .05$) decreases in plasma and RBC-AChE activity levels occurred in male and female CD rats dosed once per day, 5 d/wk, for 13 wk (see Table 22 for RBC-AChE data). Significant reductions in RBC-AChE activity relative to controls and to baseline values were seen in male rats in all dose groups. No other toxic effects were observed in the rats dosed with GB type II; however, in the study with GB type I, brain lesions (hippocampal necrosis) occurred in 1 of 12 females dosed with 0.075 mg kg^{-1} d^{-1} and in 2 of 12 females dosed with 0.3 mg kg^{-1} d^{-1}, but in none of the females dosed with 0.150 mg kg^{-1} d^{-1} and in none of the males in any dose group (Bucci et al. 1991). The absence of brain lesions at the mid-dose level, and the possibility that postmortem autolysis contributed to the findings (see Section XI.A.2, Subchronic Toxicity) makes it difficult to select a LOAEL for this endpoint. Inhibition of blood ChE is an acceptable endpoint to use in identifying a NOAEL or LOAEL; therefore, the Bucci and Parker (1992) study on GB type II is used here for the derivation of an RfD_c.

2. RfD_c Derivation. In the Bucci and Parker (1992) study, the lowest test dose of 0.075 mg GB type II kg^{-1} d^{-1}, 5 d/wk, resulted in a statistically significant reduction in RBC-AChE in male rats; therefore, this dose is considered to be a LOAEL. The LOAEL is adjusted to a 7 d/wk exposure period by using a factor of 5/7; i.e., $5/7 \times 0.075$ mg kg^{-1} $d^{-1} = 0.054$ mg kg^{-1} d^{-1}. The RfD_c is then calculated according to the following formula:

$$RfD_c = \frac{0.054 \text{ mg/kg/d}}{UF_H \times UF_A \times UF_S \times UF_L \times UF_D \times MF}$$

where:

$UF_H = 10$ (sensitive subpopulations)
$UF_A = 10$ (animal to human extrapolation)
$UF_S = 3$ (subchronic-to-chronic extrapolation)
$UF_L = 3$ (LOAEL-to-NOAEL extrapolation)

$UF_D = 3$ (data base incomplete)
MF = 1 (modifying factor, not needed)

A UF_H of 10 for sensitive subpopulations is considered necessary because some individuals have abnormally low levels of blood ChE activity that may render them especially susceptible to the effects of ChE inhibitors such as nerve agents (see Section III.B.6 for additional discussion).

An uncertainty factor of 10 is used for animal-to-human extrapolation because there is ample evidence that humans are more sensitive to GB than laboratory rodents. In humans, the single-dose oral RBC-$AChE_{50}$ (dose required to lower RBC-ChE by 50%) is 0.01 mg/kg (Grob and Harvey 1958), and an average daily dose of 0.034 mg/kg for 3 d resulted in moderate signs of toxicity. In comparison, rats receiving 0.3 mg GB type II kg^{-1} d^{-1} for 90 d exhibited decreases in blood ChE levels but no signs of toxicity (Bucci and Parker 1992).

An uncertainty factor of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of oral RfDs for other organophosphate compounds, EPA has used NOAELs for ChE inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a subchronic-to-chronic UF of 1 was used). In addition, animal data indicate that maximum ChE inhibition may occur 30–60 d or more after exposure begins, after which it levels off or even shows signs of recovery. In the Bucci and Parker study, plasma and RBC-ChE activity levels at wk 13 were no longer significantly different from either baseline or control values, particularly for the lowest dose level; therefore, increased ChE inhibition is not expected to occur at longer exposure periods. However, an uncertainty factor of 3 is used here because studies are not available to verify that adverse effects would not occur following chronic exposures. A LOAEL-to-NOAEL uncertainty factor of 3 is used instead of 10 because the endpoint, ChE inhibition, was not associated with signs of toxicity, and the LOAEL is considered to be a minimal LOAEL.

The data base for GB consists of two well-designed and well-conducted subchronic toxicity studies in rats, developmental studies in rats and rabbits, a multigeneration inhalation study in rats, delayed neuropathy studies in chickens, rats, and mice, and chronic inhalation studies in mice, rats, and dogs. In addition, there are substantial human data for acute and short-term exposures. These studies support the use of ChE inhibition as the critical endpoint for deriving an oral RfD_c. The multigeneration study was only a pilot study and gave inconclusive results because of reduced reproduction in the controls as well as in the dosed animals; therefore, a UF_D of 3 is applied.

Therefore,

$$RfD_c = \frac{0.054 \text{ mg/kg/d}}{10 \times 10 \times 3 \times 3 \times 3 \times 1}$$

$RfD_e = 0.00002$ mg GB/kg/d

$RfD_e = 0.02$ μg GB/kg/d

3. Overall Confidence in the Oral RfD_e.

Study: High
Data base: Medium
RfD_e: Medium

The principal study was well designed and well conducted, used a relevant exposure pathway, and examined the appropriate toxicological endpoints. The data base for GB also contains a second oral subchronic study in rats, chronic inhalation studies in rats, mice, and dogs, teratology studies in rats and rabbits, a multigenerational reproductive toxicity study in rats, and delayed neuropathy studies in mice and chickens.

4. Comparison of RfD_e with Human Toxicity Data. The RfD_e is compared to the available human toxicity data in Table 27. One study in humans indicated that an oral dose of 2.3 μg kg^{-1} d^{-1} for 3 d resulted in 27% and 33% RBC-AChE inhibition but no toxic effects. This dose is about 115 times greater than the derived RfD_e. For an adverse-effect level (i.e., mild toxic effect at 29 μg kg^{-1} d^{-1}) (Grob and Harvey 1958), the "margin of safety" would be about 10 times greater than that for 27%–33% RBC-AChE inhibition.

Table 27. Comparison of RfD_e with human toxicity data for agent GB.

Dose (μg/kg)	Exposure route	Endpoint	Reference
0.02[a]	Oral	RfD_e: no inhibition of RBC-AChE	This report
2	Oral	Excessive dreaming	Thienes and Haley (1972)
2.3[b]	Oral	RBC-AChE reduced 27% and 33%, but no toxic effects	Grob and Harvey (1958)
10	Oral	50% inhibition of AChE	Grob and Harvey (1958)
20	Oral	Insomnia, withdrawal, depression	Thienes and Haley (1972)
22	Oral	Mild toxic effects, anorexia, nausea, heartburn	Grob and Harvey (1958)
29	Oral	Mild toxic effects	Grob and Harvey (1958)
30	Oral	Moderate toxic effects	Grob and Harvey (1958)
34	Oral	Moderate toxic effects; > 90% reduction in RBC-AChE activity	Grob and Harvey (1958)
140	Oral	Estimated lethal oral dose	Grob and Harvey (1958)

[a]Daily dose for chronic exposure period.
[b]Average daily dose for 3 d.

XII. Agent GD (Soman)

Chemical agent GD (soman) is an organophosphate ChE inhibitor similar to other nerve agents in mode of action and toxic effects. It is toxic by all routes of administration: inhalation, ingestion, and ocular and percutaneous absorption (DA 1974). Because it is volatile, it can be a significant inhalation hazard. Animal data indicate that agent GD is similar in acute toxicity to GB but more toxic than GA. The toxicity of nerve agents has been reviewed in several earlier reports (Carnes and Watson 1989; Dacre 1984; Munro et al. 1994; Sidell 1992; Somani et al. 1992; Watson et al. 1989b). This review focuses primarily on the available subchronic and chronic oral toxicity data that may be useful for deriving an oral RfD_o. The reader is referred to the above-mentioned reports for additional information on the acute toxicity of GD.

A. Toxicology

1. Acute Toxicity. Lethality data for agent GD are summarized in Table 28. The estimated oral LD_{50} value for humans is 5–20 μg/kg (Somani et al. 1992). Gause et al. (1985) reported that the threshold for seizure induction in juvenile male baboons was 5 μg GD/kg when administered by i.m. injection. Bucci et al. (1992c) conducted range-finding studies with rats. The test material was administered by gavage to male and female CD rats once per day, 5 d/wk, for 2 wk. These studies indicated that the maximum dose tolerated by CD rats was

Table 28. Lethality data for agent GD.

Exposure route	Species	LD_{50} (μg/kg)	Reference
Oral	Human	5–20 (est.)	Somani et al. (1992)
Percutaneous	Human	50–300 (est.)	Somani et al. (1992)
Intravenous	Mouse	42	Tripathi and Dewey (1989)
	Dog	10	Abbrecht et al. (1989)
Subcutaneous	Rabbit	20	Maxwell et al. (1988)
	Guinea pig	26–30	Sterri et al. (1981); Maxwell et al. (1988); Anderson et al. (1989); Sparenborg et al. (1989)
	Rat	70–165	Somani et al. (1986); Petrali (1989); Maxwell et al. (1987, 1988); Sterri et al. (1980); Lennox et al. (1985)
	Mouse	184	Sterri et al. (1981)
Intramuscular	Monkey	~3.8–15.3	Petras (1984); Baze (1993); Wall et al. (1990); Switzer et al. (1989)
	Rabbit	15	Olson et al. (1989)
	Mouse	98	Jones et al. (1984)
Intraperitoneal	Rat	200	Sterri et al. (1980)

70 μg kg^{-1} d^{-1}, and a dose of 300 μg kg^{-1} d^{-1} was lethal to 100% of the test animals. Sterri et al. (1980, 1981) reported that repeated injections of soman in rats, guinea pigs, and mice led to cumulative LD_{50} doses markedly higher than the acute ones. The observed tolerance toward soman was thought to result from recovery of plasma aliesterase and plasma ChE (Sterri et al. 1981).

2. Subchronic Toxicity. In a subchronic study conducted by the National Center for Toxicological Research (Bucci et al. 1992c), male and female CD rats (12 per sex/group) were administered GD by gavage at dose levels equivalent to 17.5, 35.0, and 70 μg GD/kg. The doses were given once per day, 5 d/wk, for 13 wk. All animals were observed daily for clinical signs of toxicity. Necropsy examination was performed on all animals. Terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals and on those tissues of lower-dose animals that were abnormal at necropsy. Hematological analyses and clinical chemistry (including RBC- and plasma-ChE) were evaluated in the same 6 male and 6 female rats in each dose group 1 wk before the exposures began and also at wk 1, 3, 7, and 13. In addition, at necropsy a hemisection of each brain was prepared and tested for NTE.

Relative to untreated controls, the group mean body weight gain was significantly decreased ($p < .001$) in the high-dose (70 μg kg^{-1} d^{-1}) males [body weight (b.w.) change, 160.9 in controls vs. 83.7 g]. A decrease in body weight gain also occurred in high-dose female rats (59 g vs. 75 g in controls), but the difference was not statistically significant. A definitive dose response was not present in either males or females.

Although changes were observed in some clinical chemistry and hematological parameters, the changes were spurious, were not dose related, and were not biologically relevant. Brain NTE was not altered in rats dosed with GD. Histopathological examination revealed no gross or microscopic findings that could be attributed to treatment with GD. Special attention was given regarding intercostal and cardiac muscle lesions and neurological lesions that have been previously reported in rats treated with GD (McLeod 1985; Singer et al. 1987). However, none of these lesions was observed in GD-treated rats.

RBC-ChE and plasma-ChE activity levels are shown in Tables 29 and 30, respectively. Considerable variability was noted among the control and treatment group baseline values. A dose-related decrease in plasma-ChE levels in both male and female rats was observed for wk 1 and 7. Relative to untreated rats, significant ($p < .05$) depression of plasma-ChE activity was observed in both males and females of the high-dose (70 μg/kg) group during wk 1 (25% and 33% for males and females, respectively) and wk 7 (20% and 33% for males and females, respectively), and in males of the 35 μg/kg group during wk 7 (28%). In females, the plasma-ChE levels exceeded pretreatment (baseline) values by wk 13 but remained depressed (54%, 66%, and 50% in the low-, mid-, and high-dose groups, respectively) in males at wk 13, although not significantly so. On comparing treatment groups with controls, no significant changes

Table 29. RBC-ChE activity[a] in 90-day subchronic study of agent GD in CD rats.

Dose ($\mu g\ kg^{-1}\ d^{-1}$)	Sex	Week of treatment								
		−1	1	%[b]	3	%[b]	7	%[b]	13	%[b]
0	F	1609 (252)	2461 (136)[c]	153	2497 (186)[c]	155	1674 (127)	104	1341 (397)	83
17.5	F	1462 (153)	2445 (191)[c]	167	1790 (144)[d]	122	1236 (201)[c]	85	1313 (280)	89
35.0	F	1466 (167)	2244 (179)[c]	153	1817 (127)[d]	124	1381 (90)	94	1310 (280)	89
90.0	F	1025 (152)	2544 (367)[c]	250	1695 (52)[c,d]	165	1556 (96)	152	1296 (168)	126
0	M	1955 (108)	2340 (149)	119	2043 (212)	105	2323 (116)	119	1904 (106)	97
17.5	M	1764 (158)	1862 (95)	106	1211 (125)[c,d]	69	1784 (90)[d]	101	1774 (161)	100
35.0	M	1977 (78)	1820 (153)[d]	92	1435 (320)[c]	73	1681 (28)[d]	85	1562 (68)	79
90.0	M	1859 (142)	1964 (205)	105	973 (48)[c,d]	52	1753 (139)[d]	94	1720 (148)	92

[a]Values given as mean IU/L and (SEM).

[b]Percent of baseline.

[c]Significantly different ($p < .05$) relative to preexposure value (wk −1) based on ANOVA and Dunnett's and Scheffe's comparisons (ORNL).

[d]Significantly different ($p < .05$) relative to weekly controls based on ANOVA and Dunnett's and Scheffe's comparisons (ORNL).

Source: Bucci et al. (1992c).

Table 30. Plasma-ChE activity[a] in 90-day subchronic study of agent GD in CD rats.

Dose ($\mu g\ kg^{-1}\ d^{-1}$)	Sex	Week of treatment								
		−1	1	%[b]	3	%[b]	7	%[b]	13	%[b]
0	F	1401 (148)	1542 (175)	110	437 (52)[d]	31	1976 (198)	141	2659 (358)[d]	190
17.5	F	1571 (322)	893 (185)[c]	57	1131 (422)	72	968 (109)[c]	61	2275 (320)	145
35.0	F	1560 (207)	593 (50)[d,c]	38	337 (80)[d]	22	673 (96)[d,c]	43	2303 (179)[d]	148
90.0	F	1344 (190)	446 (81)[c,d,e]	33	950 (180)	71	439 (40)[c,d,e]	33	1951 (179)[d]	145
0	M	543 (51)	377 (44)	69	685 (290)	126	340 (18)	63	431 (39)	78
17.5	M	631 (131)	245 (29)[c]	39	500 (293)	79	204 (19)[d]	32	339 (34)	54
35.0	M	632 (47)	198 (16)[d,c]	31	276 (63)[d]	44	174 (14)[c,d,e]	28	414 (32)[d]	66
90.0	M	610 (98)	153 (11)[c,d,e]	25	370 (59)[d]	61	122 (7)[c,d,e]	20	308 (16)[d,c]	50

[a]Mean IU/L and (SEM).

[b]Percent of baseline (wk −1).

[c]Significantly ($p < .05$) different from control value (0 µg/kg) (Bucci et al. 1992c).

[d]Significantly different ($p < .05$) relative to preexposure value (wk −1), based on ANOVA and Dunnett's and Scheffe's comparisons (ORNL).

[e]Significantly different ($p < .05$) relative to weekly controls, based on ANOVA and Dunnett's and Scheffe's comparisons (ORNL).

Source: Bucci et al. (1992c).

in RBC-AChE levels were noted by the study authors. Data for wk 3 were highly variable and appear to reflect inaccuracies or problems with the AChE assay procedure.

The plasma- and RBC-ChE data from the Bucci et al. (1992c) study were reanalyzed statistically (using SD) with ANOVA and Dunnett's and Scheffe's comparisons. In the reevaluation, RBC- and plasma-ChE activity levels were compared to respective controls for the same sampling times as well as to the baseline values within each group. This analysis also indicated an absence of definitive changes in RBC-ChE activity that could be attributed to GD treatment. During wk 3 in females and wk 7 in males, RBC-ChE activity of all GD treatment groups was significantly lower ($p < .05$) than controls, but the response did not exhibit a dose relation in either group. In females during wk 1, RBC-ChE levels in the control and all treatment groups were inexplicably elevated relative to baseline (wk −1) values. For plasma-ChE, a dose-related decrease ($p < .05$) relative to controls was detected during wk 1 and 3 for both male and female rats. With the exception of high-dose females at wk 3, a comparison of values to baseline indicated that plasma-ChE activity levels of both the mid- and high-dose groups were significantly ($p < .05$) lower for wk 1, 3, and 7 for females and throughout the 13-wk period for males. Under the conditions of this study, GD treatment appeared to affect plasma-ChE levels at a dose as low as 17.5 μg/kg, as exemplified by the significant ($p < .05$) decrease relative to controls and baseline values in male (39% of baseline) and female (57% of baseline) rats at wk 1. For plasma-ChE, decreases in activity levels appeared to be dose related. It is uncertain why similar findings were not observed for RBC-ChE.

Dulaney et al. (1985) evaluated the effects of GD on the growth rates of rats given daily subcutaneous doses of 25 μg/kg for 85 d. The dosed animals exhibited reduced growth rates (68% of controls in the first 20 d, 60% of controls in the next 10 d, and 95% of controls after the 30th d). AChE activity was determined in the striatum and the remainder of the brain 24 hr after the last exposure. Mean brain striatal AChE activity was only 35% of the control value. In cumulative mortality studies, rats were dosed with 25 μg kg^{-1} d^{-1} for 85 d or 35, 40, or 50 μg kg^{-1} d^{-1} for 25 d; mortality ranged from 0 in the two low-dose groups to two of eight in the 40 μg/kg group to 100% in the high-dose group.

3. Chronic Toxicity. Information on the toxicity of agent GD to humans or animals following chronic exposures was not found in the available literature.

4. Neurotoxicity. Animal studies indicate that acute and subacute exposures to GD can result in neurobehavioral changes; however, few studies have evaluated potential neurological effects following long-term low-level exposures. Neurobehavioral effects (attention deficits) have been reported in juvenile male baboons following a single exposure to 4–5 μg GD/kg by i.m. injection or 3 μg/kg followed by 1 μg kg^{-1} wk^{-1} for 4 subsequent weeks (Gause et al. 1985). The

highest dose tested resulted in seizures in some of the test animals. The effects of GD on performance of a compensatory tracking task by rhesus monkeys was assessed by Blick et al. (1994), who reported an ED_{50} of 0.97 μg/kg for 5 d of daily parenteral injections. The ED_{50} dose was associated with an 85%–90% inhibition of serum ChE. Marmosets injected i.m. with 1.75 μg GD/kg exhibited deficits in hand–eye coordination tests (Wolthuis 1992). Rats injected s.c. with 100–110 μg GD/kg exhibited neuropathology and deficits in performance of operant tasks (McDonough et al. 1986). Intraperitoneal doses as low as 10 μg GD/kg suppressed schedule-control behavior in rats without overt signs of toxicity; however, weekly injections resulted in tolerance to the response (i.e., less suppression of the behavior) (Hymowitz et al. 1985). In contrast, repeated s.c. injections of 60 μg GD/kg (3 times/wk for up 6 wk) in rats did not induce behavioral tolerance as measured in tests of avoidance response (van Dongen and Wolthuis 1989; Wolthuis et al. 1990). Intraperitoneal doses of 4 or 20 μg/kg resulted in dose-dependent decreases in rearing and ambulation and increases in nonmobile exploration in male Wistar rats (Nieminen et al. 1990). Four injections of 46 μg GD/kg, given every 3 d during a 2-wk period, were sufficient to prevent rats from learning an avoidance-escape response; four of seven animals dosed with 31 μg/kg learned the task (Geller et al. 1985). Subcutaneous injections of 12.7 or 25.5 μg GD/kg, given 5 d/wk for 4 wk, resulted in a dose-dependent reduction in avoidance behavior efficiency in male Sprague-Dawley rats (Geller et al. 1987). Blood ChE was inhibited as much as 57% after the low dose and 74% after the high dose. Behavior returned to control levels after the exposure was discontinued. Subcutaneous injections of 0.35 μg GD kg^{-1} d^{-1} for 3 d, followed by the same dose 3 times/wk for a total of 11 injections, resulted in no overt signs of toxicity in Sprague-Dawley rats; however, differential effects were observed in enzymatic, physiological, and behavioral functions (Russell et al. 1986). RBC-AChE activity levels were reduced to about 30% or less of the control values and brain AChE to 35%–70% of the control values during the exposure period. Sensory and perceptual thresholds were elevated (hypalgesia) throughout the exposure period. Temporal perception (as evidenced in fixed interval responding) was significantly impaired initially, but tolerance developed during the exposure period. Deficits in motor and cognitive functions also showed recovery.

Evidence of organophosphate-induced delayed neuropathy was not observed in rats dosed with agent GD by gavage once per day for 90 d (Bucci et al. 1992c), nor in cats dosed s.c. with either a single dose of 1 mg GD/kg, with 2.5 μg kg^{-1} d^{-1} for 10 d, or with 10 μg kg^{-1} d^{-1} for 5 d (Goldstein et al. 1987). Two delayed neurotoxicity studies in chickens also gave negative results (Bucci et al. 1992d; Gordon et al. 1983). Although agent GD produced signs of delayed neuropathy in one antidote-protected chicken surviving an i.m. injection of 1.5 mg GD/kg, about 150 times the LD_{50} (Willems et al. 1984), the results of this study were considered questionable because it was later determined by the same researchers that although GD inhibits NTE, the inhibited NTE does not "age" to the form responsible for causing delayed neuropathy (Johnson et al. 1988).

5. Developmental and Reproductive Effects. No studies evaluating the developmental or reproductive effects of GD in humans or laboratory animals were located in the available literature.

6. Carcinogenicity. No information is available regarding the potential carcinogenicity of agent GD in humans. In a 90-d gavage study in rats, Bucci et al. (1992c) reported no neoplastic changes attributable to GD treatment. The study was, however, of insufficient duration to be suitable for a cancer bioassay. No additional data are available regarding the potential carcinogenicity of GD in animals.

7. Genotoxicity. Goldman et al. (1987) reported on the results of genotoxicity studies of agent GD. In tests on bacteria and mammalian cell cultures, GD was not genotoxic or mutagenic when tested with and without metabolic activation. There were no biologically significant increases in mutations when tested in the Ames *Salmonella* assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) either with or without metabolic activation. GD did not induce a significant increase in forward mutations when tested on mouse L5178Y lymphoma cells at concentrations of 50, 100, or 200 μg/mL, and no increase in SCE was observed when Chinese hamster ovary cells were exposed *in vitro* to 200 μg/mL of GD. Mice treated *in vivo* with a maximally tolerated i.p. dose of 300 μg GD/kg did not exhibit a significant increase in SCE in splenic lymphocytes. Exposure of rat hepatocytes to GD concentrations as high as 600 μL/3 ml culture medium (2.5×10^6 hepatocytes) did not result in DNA damage or unscheduled DNA synthesis (see also Klein et al. 1987).

B. Estimated Reference Dose

1. Selection of the Key Study. In the derivation of an oral RfD, long-term human oral exposure data are preferred; however, such data are not available for agent GD. The only available subchronic or chronic exposure study on agent GD consists of a 90-d study in which rats were administered the agent by gavage (Bucci et al. 1992c). A 90-d rodent study is of sufficient duration to be considered a subchronic exposure in deriving an oral RfD_e. Briefly summarized, the results of this study showed statistically significant ($p < .05$) decreases in plasma-ChE activity levels in male and female rats dosed with 17.5 μg GD kg^{-1} d^{-1}, 5 d/wk, for 13 wk. There were no definitive dose-related changes in RBC-ChE, and NTE levels were not significantly affected by the GD treatment.

2. RfD_e Derivation. The lowest tested dose (17.5 μg kg^{-1} d^{-1} = 0.0175 mg kg^{-1} d^{-1}) in the Bucci et al. (1992c) study is considered a LOAEL because of the statistically significant reduction (relative to controls and to baseline values) in plasma-ChE during wk 1. This dose is adjusted to a 7 d/wk exposure period by using a factor of 5/7; i.e., $5/7 \times 0.0175$ mg kg^{-1} d^{-1} = 0.0125 mg kg^{-1} d^{-1}. The RfD_e can then be calculated according to the following formula:

$$\text{Oral RfD}_e = \frac{0.0125 \text{ mg/kg/d}}{UF_H \times UF_A \times UF_S \times UF_L \times UF_D \times MF}$$

where:

$UF_H = 10$ (sensitive subpopulations)
$UF_A = 10$ (animal to human extrapolation)
$UF_S = 3$ (although plasma-ChE is not expected to be inhibited at longer exposures, however, an uncertainty factor was incorporated to account for effects possibly unrelated to plasma-ChE inhibition)
$UF_L = 3$ (LOAEL to NOAEL extrapolation; altered plasma-ChE is not overtly toxic)
$UF_D = 3$ (data base incomplete because of lack of a toxicity study in a second species, as well as reproductive and developmental toxicity studies
$MF = 1$ (no additional modifications needed)

A UF_H of 10 for sensitive subpopulations is considered necessary because some individuals have abnormally low levels of blood ChE activity that may make them especially susceptible to the effects of ChE inhibitors such as nerve agents (see Section III.B.6 for additional discussion). An uncertainty factor of 10 is used for animal-to-human extrapolation because there is no evidence suggesting that humans are less sensitive to GD than are laboratory animals. An uncertainty factor of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of the oral RfDs for other organophosphate compounds, the EPA has used NOAELs for ChE inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a subchronic-to-chronic UF of 1 was used). In addition, animal data indicate that maximum ChE inhibition may occur 30–60 d or more after exposure begins, after which it levels off or even shows recovery. In the Bucci et al. (1992c) study, both plasma and RBC-AChE levels exhibited signs of recovery at wk 13, especially for the lower doses (see Tables 29, 30). Therefore, increased ChE inhibition is not expected to occur at longer exposure periods. However, an uncertainty factor of 3 is used because studies are not available to verify that adverse effects would not occur following chronic exposures.

A LOAEL-to-NOAEL uncertainty factor of 3 is used instead of 10 because the endpoint, ChE inhibition, was not associated with signs of toxicity.

The database for GD lacks chronic oral studies in two species and studies assessing reproductive/developmental effects. Because studies on other organophosphate ChE inhibitors, including a multigeneration study on agent VX, indicate that reproductive/developmental effects are unlikely, a full uncertainty factor of 10 is not warranted.

Therefore,

$$\text{Oral RfD}_e = \frac{0.0125 \text{ mg/kg/d}}{10 \times 10 \times 3 \times 3 \times 3}$$

$$\text{Oral RfD}_e = 0.000004 \text{ mg GD/kg/d}$$

$$\text{Oral RfD}_e = 0.004 \text{ } \mu\text{g GD/kg/d}$$

3. Overall Confidence in the RfD_e.

Study: Medium
Data base: Low
RfD: Low to Medium

The principal study was well designed and well conducted, used a relevant exposure pathway, and examined the appropriate toxicological endpoints; however, there was considerable variability in the blood ChE values of the exposed animals. The data base for GD lacks a toxicity study in a second species, chronic studies, as well as reproductive and developmental toxicity studies, including a multigenerational study. The unlikelihood that agent GD is a reproductive or developmental toxin reduces that significance of these data gaps. Overall confidence in the RfD_e is low to medium.

4. Comparison of RfD_e with Toxicity Data. Only limited data regarding exposure to GD are available for comparison to the proposed RfD_e. An oral LD_{50} of 5–20 mg/kg for humans was estimated by Somani et al. (1992). The proposed RfD_e of 0.004 μg/kg is lower by a factor of 10^6. The proposed RfD_e is more than two orders of magnitude below the ED_{50} dose (0.97 μg/kg) shown to produce performance decrements in rhesus monkeys after 5 consecutive d of dosing (Blick et al. 1994). Nieminen et al. (1990) reported behavioral effects concurrent with reduced blood AChE levels in rats given single i.p. injections of GD at doses of 4 or 20 μg/kg.

XIII. Agent L (Lewisite)

Lewisite is a lethal vesicant and systemic poison. The acute toxicology of lewisite has been reviewed by Goldman and Dacre (1989), Watson and Griffin (1992), and Trammell (1992) and, therefore, is only briefly discussed here.

A. Toxicology

Lewisite may be lethal following inhalation or dermal exposure, or by ingestion. Its lethality results primarily from vapor inhalation, although lewisite is much less potent than neurotoxic chemical warfare agents. Generally, the toxic effects of lewisite are of rapid onset and result from acute exposures. The vesicant properties of lewisite result from direct skin contact; it has been estimated that as little as 2 mL to an adult human (equivalent to 37.6 mg/kg) can be fatal within several hours (Sollman 1957). Because it is lipophilic, percutaneous absorption of lewisite is rapid and may be associated with systemic toxicity characterized by pulmonary edema, diarrhea, agitation, weakness, hypothermia, and hypotension (Institute of Medicine 1993). The threshold for severe systemic effects in humans following dermal exposure to lewisite is approximately 10

mg/kg (9.1–13.4 mg/kg) (Sollman 1957). It has been hypothesized that fatalities following dermal exposure to lewisite may result from blood plasma loss resulting from extensive capillary damage (i.e., lewisite shock) (Cameron et al. 1946). Ingestion of trivalent arsenicals may also cause death from fluid loss resulting from intestinal epithelium damage. The vesicant properties of lewisite are characterized by immediate onset of pain and, for ocular exposure, possible corneal necrosis. Studies in animals have shown that the target tissues and organs for systemic toxicity of lewisite include the liver, gallbladder, urinary bladder, lung, and kidneys (Cameron et al. 1946; Snider et al. 1990). It is important to note that the gaps in knowledge regarding the toxic effects and dose response for lewisite are extensive.

1. Acute Toxicity. Liquid lewisite applied by eye-dropper to the forearms of men caused blanching and discoloration of the skin followed by extensive erythema within 15–30 min and vesication within 12 hr (Wardell 1941; as cited in Goldman and Dacre 1989). The pain associated with these dermal exposures reportedly occurred within 2 min, and considerable discomfort persisted for about 1 wk. Other tests with human subjects and clinical reports also indicate a similar temporal sequence of events. Exposure to lewisite vapor (0.06–0.33 mg/L) caused discoloration and blistering, with the maximum effect occurring by 36–48 hr after exposure (Wardell 1941). At a concentration of 0.01 mg/L, lewisite vapor caused inflammation of the eyes and swelling of the eyelids after 15 min of exposure, and inhalation of 0.5 mg/L for 5 min is considered to be potentially lethal.

Short-term exposure (10–30 min) of dogs to lewisite vapor (0.05–0.12 mg/L) produced vomiting, urination, defecation, and severe respiratory distress that resulted in the death of 80% of the dogs within 3–48 hr (Goldman and Dacre 1989). It was not reported whether the exposures were whole body or head only.

Acute oral toxicity values for lewisite have been summarized by Watson and Griffin (1992). The only available oral LD_{50} is that for the rat (50 mg/kg). Lethality values for other routes of exposure indicate some species variability, but the values differ by less than an order of magnitude for any particular exposure route.

2. Subchronic Toxicity. A drinking water exposure study in rats was reported by Leitch et al. (1941). In this study, 10 rats were administered lewisite in drinking water (10 or 16 mg/L) for 19 wk (133 d). The treatment did not affect consumption of food or water and had no effect on animal growth. Additionally, there were no treatment-related histopathological findings. Based on this report, a lewisite concentration of 16 mg/L drinking water would represent a NOAEL. However, this study has some deficiencies, as noted by Daniels (1990). The study neither defined an effect level nor monitored the actual concentration of lewisite in drinking water consumed. Additionally, the report did not provide information regarding water consumption by the test animals. Such data would be critical in determining an actual or estimated dose of lewisite. It is also

possible that the consumed concentration may have varied from the target concentration because of test article degradation. Daniels (1990), however, suggested that these data would probably provide an estimate of a 7-d NOEL equivalent to 1.4 mg lewisite/kg.

In a dose range-finding study for a teratology study in rats and rabbits, lewisite was administered by gavage to rats (10/group) on gestation d 6–15 at doses of 0, 0.5, 1.0, 2.0, or 2.5 mg/kg, and by gastric intubation to rabbits (8/group) on gestation d 6–19 at doses of 0, 0.5, 1.0, 1.5, or 2.0 mg/kg. For rats, deaths attributed to lewisite occurred in the 2.5 mg/kg group (2/10) and in the 2.0 mg/kg group (1/10). Dosing trauma deaths were also reported (1/10, 2/10, and 1/10 in the 1.0, 2.0, and 2.5 mg/kg groups). For rabbits, deaths attributed to lewisite were reported in the 1.0 mg/kg group (6/8), 1.5 mg/kg group (5/8), and 2.0 mg/kg group (8/8). Dosing trauma deaths were also noted, 1/8 and 3/8 in the 1.0 mg/kg group and 1.5 mg/kg group, respectively.

A 90-d subchronic toxicity study of lewisite in rats was conducted by Sasser et al. (1989c, 1996b). In this study, groups of 10 male and 10 female rats were given lewisite in sesame oil by gastric intubation at doses of 0.01, 0.1, 0.5, 1.0, or 2.0 mg/kg. Dosing protocol was 5 d/wk for 13 wk. Vehicle controls received sesame oil at a dose of 1.67 mL/kg. Deaths were observed in the three highest dose groups: 3 males and 7 females of the 2.0 mg/kg dose groups, 8 males and 6 females of the 1.0 mg/kg dose group, and 2 males and 3 females of the 0.5 mg/kg dose group. Although all the deaths occurred in the three highest dose groups, the response was not dose dependent. Forestomach lesions were observed in the two highest dose groups (8/10 males and 4/10 females in the 2.0 mg/kg group, and 1/10 males in the 1.0 mg/kg group) and were attributed to the test article. These lesions were characterized by necrosis of the stratified squamous epithelium accompanied by infiltration of numerous neutrophils and macrophages, hemorrhage, and edema. In some instances, hyperplasia of adjacent areas was noted. There was no evidence that the lesions were precancerous, but the duration of exposure and observation was insufficient to assess carcinogenic responses. Lesions were also present in the glandular stomach but to a lesser degree. The presence of the lesions was consistent with the irritant effect of lewisite. No lesions were observed in the lower dose groups.

Some of the animals died without exhibiting any clinical signs of toxicity; drooling or wetness around the mouth and chin, and labored respiration were noted among other rats immediately preceding death. Gross pathology findings attributed all deaths, except one, to severe inflammatory lesions characterized by edema and epithelial necrosis of the respiratory tract. Respiratory lesions were most likely caused by aspiration of the test material or induced reflux of stomach contents into the pharynx with subsequent aspiration into the airway. Inflammatory lesions observed in the respiratory tract of surviving rats were also indicative of accidental deposition or induced reflux of the test material. No significant treatment-related effects on body weights or organ weights were observed for any of the dose groups.

Clinical chemistry evaluations revealed a significant ($p < .05$) decrease in

total serum protein, serum creatinine, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) in male rats of the highest dose (2.0 mg/kg) group at 13 wk. Lowered serum enzyme activity was also observed in male rats of the other lewisite dose groups. Females of the highest dose group exhibited significantly increased lymphocyte and platelet counts, the former at 6 wk but not at 13 wk and the latter only at 13 wk. The biological and toxicological significance of these findings is, however, uncertain. The investigators noted that the no-effect dose was greater than 0.5 mg/kg and less than 1.0 mg/kg. The 0.5 mg/kg dose may be considered an estimate of the NOAEL for short-term oral exposure to lewisite.

3. Chronic Toxicity. No human or animal studies examining the effects of lewisite following chronic exposure were located in the searched literature.

4. Developmental and Reproductive Effects.

Human Data. Human data regarding reproductive/developmental effects from lewisite exposure are inconclusive because of confounding factors, including concurrent exposure to other agents such as sulfur mustards and incomplete exposure data. Yamakido et al. (1985) studied workers from the Okuna-jima (Japan) factory where mustard and lewisite were manufactured in the World War II era and noted no evidence of agent-induced mutations.

Animal Studies. In a teratogenicity study by Hackett et al. (1987), lewisite was administered by gavage to pregnant rats on gestation d 6–15 at doses of 0.5, 1.0, or 1.5 mg/kg and by gastric intubation to pregnant rabbits on gestation d 6–19 at doses of 0.07, 0.2, or 0.6 mg/kg. For rabbits, the mortality rates were 13%, 46%, and 69% for the 0.07, 0.2, and 0.6 mg/kg dose groups, respectively. The mortality rates were corrected for death from other causes (e.g., dose-delivery trauma, accidental delivery of the dose to the lungs, handling trauma, pregnancy complications unrelated to the test article) and, therefore, represent a significant dose-related frank effect. Surviving rabbits in the highest dose group exhibited decreased body weight gain relative to controls and other dose groups. However, the study authors noted more frequent incidences of anorexia in the high-dose rabbits when compared to controls and other dose groups. For those rabbits whose deaths were not attributed to the extraneous causes previously noted, gastric lesions (mucosal inflammation, edema, necrosis, and mucosal sloughing) were observed at all dose levels. The only statistically significant developmental effects were significant increases in the incidence of fetal stunting and supernumerary ribs in the high-dose (0.6 mg/kg) group. Fetal weight and crown–rump length were somewhat lower in the 0.6 mg/kg dose group, but these differences were not statistically significant. Maternal toxicity (13%) was also associated with the low-dose group, thereby indicating a NOAEL for this study to be <0.07 mg kg^{-1} d^{-1} for maternal toxicity and 0.2 mg kg^{-1} d^{-1} for developmental toxicity. The LOAEL based on maternal effects is 0.07 mg kg^{-1}

d^{-1} and for developmental effects is approximately 0.6 mg kg^{-1} d^{-1}. The increased mortality of the does (13%) and the occurrence of gastric lesions in the low-dose group (0.07 mg kg^{-1} d^{-1}) suggest that the rabbit is the most sensitive of the species for which data are available.

The use of increased mortality as the critical effect for derivation of a reference dose is not appropriate. Furthermore, the intragastric intubation technique used for rabbits in this study concentrates the test article on the gastric mucosa more effectively than simple gavage administration, thereby making the apparent increased sensitivity of rabbits more an artifact of administration than actual toxicodynamics. Hackett et al. (1987) noted that the fetal toxicity observed in the rabbits appeared to be occurring at doses greater than those required to induce increased maternal mortality. The findings of this study are, however, statistically compromised by the low number of pregnant survivors (9/12, 6/11, 5/13, and 3/15 for control, 0.01, 2.0, and 0.6 mg/kg dose groups, respectively). In a dose range-finding study for this experiment (see Section XIII.A.2, Subchronic Toxicity), significant mortality was observed in the 1.0 mg/kg (6/8), 1.5 mg/kg (5/8), and 2.0 mg/kg (8/8) groups.

Another phase of the Hackett et al. (1987) study investigated the potential teratogenicity of lewisite in rats. In this phase of the study, no maternal toxicity or teratogenic effects were observed, thereby identifying 1.5 mg/kg as a NOAEL. However, it must be noted that in a dose range-finding study in rats (Hackett et al. 1987) (see Section XIII.A.2, Subchronic Toxicity), doses of 2.0 mg/kg and 2.5 mg/kg resulted in 10% and 20% maternal mortality, respectively.

A two-generation reproductive study in rats was conducted by Sasser et al. (1989d). Lewisite (in sesame oil) was administered intragastrically at doses of 0.10, 0.25, or 0.60 mg kg^{-1} d^{-1} to groups of 25 male and 25 female rats, 5 d/wk, for 13 wk before mating, and 7 d/wk during gestation (21 d), and at least 4 d/wk during lactation (21 d). The doses were selected on the basis of the findings of the subchronic toxicity study by Sasser et al. (1989c), which identified a NOAEL between 0.5 and 1.0 mg kg^{-1} d^{-1}, and the teratogenicity study by Hackett et al. (1987) in which 1.5 mg kg^{-1} d^{-1} was a NOAEL. In the dose range-finding phase of this study, 20% mortality (corrected for deaths from dosing trauma) was observed in the 2.5 mg kg^{-1} d^{-1} group. At the time of birth of the F_1 generation, the F_0 male rats were killed. Dams continued treatment (minimum of four doses per week) throughout lactation (3 wk). A vehicle control group was given equivalent volumes of sesame oil (1.67 ml/kg). After weaning, 20 male and 25 female offspring were selected for the F_1 phase of the study. The treatment protocol for these animals was as described for the F_0 generation. Mortality was high among both the F_0 and F_1 females. The cause of death for most of these animals appeared to be associated with aspiration of the test article, resulting in fatal respiratory tract lesions. Exposure of rats to lewisite did not adversely affect reproductive performance, fertility, or reproductive organ weights. The treatment had no significant effect on litter weights, sex ratio, mean pup weight, or offspring survival for either generation. Although this

study revealed no toxic effects, arsenic is known to be embryotoxic and teratogenic, and the possibility exists that inorganic arsenic could be metabolically derived from lewisite.

An unpublished U.S.S.R. study analyzed by the U.S. Army Research Institute of Chemical Defense (Solana 1992) provided data indicating that preconception maternal exposure of rats to 0.045 or 0.002 mg lewisite/cm^3, 4 hr/d, 5 d/wk for 4 mon, did not affect numbers of corpora lutea or implantations, number and physical dimensions of fetuses, increased intrauterine mortality, or ossification of long bones. Approximately 140 litters of rats were used in this study.

5. Carcinogenicity. In a long-term follow-up study, Krause and Grussendorf (1978) reported the formation of a malignant lesion at the site of contact 8 yr after a single, acute dermal exposure to lewisite. A German soldier had been accidentally exposed to liquid lewisite on his lower right leg in 1940. In 1948, the lesion was diagnosed as malignant, and 38 yr after exposure, the area around the contact site was still ulcerated and was diagnosed as Bowen's disease (intradermal squamous cell carcinoma). Bowen's disease was also diagnosed in workers at a Japanese facility that produced lewisite (Inada et al. 1978). These latter findings, however, were not conclusive because these workers were exposed concurrently to diphenylcyanoarsine and mustard agent and no quantitative estimates of dose or exposure rates were available (Inada et al. 1978).

There is only anecdotal evidence for the potential carcinogenicity of lewisite. These data are not definitive and do not support classifying lewisite as a suspected carcinogen. As such, quantitative assessment of the potential carcinogenicity of lewisite is not currently possible. Although the available evidence is not of sufficient quality to label lewisite a suspected carcinogen, the position maintained by Centers for Disease Control (CDC) (DHHS 1988) that "some evidence suggests that lewisite might also be a carcinogen" seems tenable. However, for environmental exposure and remediation concerns, the arsenic component or arsenic-containing degradation products would warrant concern.

Although the carcinogenicity of lewisite *per se* is equivocal and cannot be assessed quantitatively, several of its degradation products are known carcinogens. Lewisite combustion produces the inorganic arsenicals arsenic trichloride and arsenic trioxide, as well as vinyl chloride. Inorganic arsenic is carcinogenic in humans and animals and is classified as a Group A carcinogen for both oral and inhalation exposure (USEPA 1994b). Arsenic trioxide and vinyl chloride are both considered Group A carcinogens by the USEPA (USEPA 1984a, 1988) and Group 1 carcinogens by IARC (IARC 1987b). Additionally, compounds such as arsenic trichloride, sodium arsenite (a lewisite hydrolysis product), arsenic oxychloride, and inorganic arsenicals in general are of concern to EPA as potential carcinogens (USEPA 1988). However, there are no human epidemiological data or data from animal studies that show organic arsenicals to be carcinogenic. A review by the World Health Organization (WHO 1981) stated that "There is no conclusive evidence that any of the organoarsenic compounds

tested for carcinogenicity in laboratory animals are carcinogenic." IARC (1987b) concluded that adequate data were not available for evaluating the carcinogenicity of organic arsenic compounds.

6. Genotoxicity. Data from genotoxicity studies do not indicate a carcinogenic potential for lewisite. Genotoxicity studies in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 were negative with and without S9 activation at lewisite concentrations less than 1.0 μg/plate (Stewart et al. 1989b). At 1.0 μg/plate and higher, lewisite was cytotoxic. Jostes et al. (1989b) reported on the effects of lewisite in one mutation assay [hypoxanthine-guanine phyosphoribosyl transferase (HGPRT) locus] and two cytogenetic assays [chromosomal aberration and sister chromatid exchange (SCE)] using Chinese hamster ovary cells. At concentrations ranging from 0.12 to 2.0 μM, the mutagenic response at the HGPRT locus was not significantly different from control values. The SCE assay resulted in a weakly positive response with concentrations from 0.25 to 1.0 μM, but the values were not significantly different from control values. However, chromosome aberrations were induced at 0.50, 0.75, and 1.0 μM that were significantly greater than control values. The investigators concluded that lewisite was cytotoxic and clastogenic but that SCE and mutation at the HGPRT locus were insignificant. Assays to determine sex-linked lethal mutations and chromosomal rearrangements in *Drosophila melanogaster* yielded negative results (Auerbach and Robson 1946, 1947b).

A dominant lethal study using CD rats was conducted by Bucci et al. (1993b). In this study, male CD rats (20/group) were given lewisite in sesame oil by gavage for 5 d at doses of 0.375, 0.75, or 1.5 mg/kg. Vehicle controls received an equivalent volume of the vehicle, and positive controls were given the vehicle followed by 100 mg ethyl methanesulphonate/kg i.p. on d 5. Each male was mated with two females during the next 10 wk. With the exception of the positive controls, no significant differences were observed in reproductive indices, and there were no histopathological findings that could be attributed to lewisite treatment. Under the conditions of this study, no dominant lethal mutations resulted from exposure to lewisite.

B. Estimated Reference Dose

1. Selection of the Key Study. The effects levels for the available studies are summarized in Table 31. A NOAEL between 0.5 and 1.0 mg kg^{-1} d^{-1} was obtained from the rat 90-d oral subchronic toxicity study of Sasser et al. (1989c). The oral teratogenicity study by Hackett et al. (1987) provided data indicating a NOAEL of 1.5 mg/kg for teratogenic effects in rats, with maternal toxicity occurring at 2.0 mg kg^{-1} d^{-1}. Hackett et al. (1987) also reported that gestational exposure of rabbits at 0.6 mg kg^{-1} d^{-1} resulted in maternal toxicity and fetal stunting. Doses as low as 0.07 mg kg^{-1} d^{-1} also resulted in 13% maternal toxicity (excluding deaths from extraneous causes) and were accompanied by marked gastric lesions. These data indicate a LOAEL of 0.07 mg/kg based on gastric

Table 31. Summary of effect levels for lewisite toxicity studies.

Study type[a]	Species	NOAEL	LOAEL (critical effect)	Reference
Subchronic	Rat	1.4 mg/kg (est.)	None	Leitch et al. (1941)
90-d	Rat	0.5 mg kg^{-1} d^{-1}	1.0 mg $kg^{-1}d^{-1}$ (gastric lesions)	Sasser et al. (1989c)
Multigeneration	Rat	0.6 mg $kg^{-1}d^{-1}$ (0.44 mg $kg^{-1}d^{-1}$ TWA)	None	Sasser et al. (1989d)
Developmental[b]	Rat	1.5 mg $kg^{-1}d^{-1}$	None	Hackett et al. (1987)
Developmental[b]	Rabbit	<0.07 mg $kg^{-1}d^{-1}$	0.07 mg $kg^{-1}d^{-1}$ (gastric lesions, increased mortality)	Hackett et al. (1987)
Range-finding[b]	Rat	1.0 mg $kg^{-1}d^{-1}$	2.0 mg $kg^{-1}d^{-1}$ (increased mortality)	Hackett et al. (1987)
Range-finding[b]	Rabbit	0.5 mg $kg^{-1}d^{-1}$	1.0 mg $kg^{-1}d^{-1}$ (increased mortality)	Hackett et al. (1987)

[a]Route of administration is gavage/gastric intubation, except Leitch et al. (1941), which was drinking water.

[b]Test article administered on gestation d 6–15 (rats) and 6–19 (rabbits).

lesions and increased mortality. However, the results of this study are statistically compromised by the low numbers of surviving animals. A NOAEL of 0.6 mg/kg was obtained from the multigeneration reproduction study in rats reported by Sasser et al. (1989d). Although the data from the 90-d subchronic toxicity study by Sasser et al. (1989c) only identified the NOAEL as between 0.5 and 1.0 mg/kg, the 0.5 mg/kg dose would provide a conservative estimate of the NOAEL.

On the basis of the limited data available, the rabbit appears to represent the most sensitive species as indicated by the occurrence of gastric lesions concurrent with increased mortality following 14-d administration of lewisite by gastric intubation. The rabbit data are, however, statistically compromised by the small number of survivors in each treatment group. Studies assessing reproductive or developmental endpoints in rats were negative, and the data from rabbits indicated that developmental effects occurred at doses exceeding those that induce significant maternal mortality.

For the derivation of an RfD_c for lewisite, both a 90-d study (Sasser et al. 1989c) and a multigeneration study (Sasser et al. 1989d) in rats were used to

identify effect levels. Data from the 90-d study identified a LOAEL of 1.0 mg kg^{-1} d^{-1} based on gastric lesions. The accompanying NOAEL from this study was 0.5 mg kg^{-1} d^{-1}. The multigeneration study represents a chronic exposure situation relative to reproductive/developmental effects but would be considered subchronic duration for systemic effects in the adult animals. It must be noted that the absence of reproductive/developmental effects does not necessarily eliminate the possibility of more sensitive effects in alternate targets. The highest dose (0.6 mg kg^{-1} d^{-1}) from the multigeneration study of Sasser et al. (1989d) appears to represent the most valid NOAEL and would be the best value for deriving an RfD_c.

2. RfD_c Derivation. Because of the discontinuous exposure and variable dosing protocol used in the study by Sasser et al. (1989d), a time-weighted average dose must be calculated for study protocol. This adjustment provides a NOAEL adjusted for discontinuous exposure ($NOAEL_{adj}$) and is based on the following: rats were dosed at 0.6 mg kg^{-1} d^{-1} × 5 d/7 d for 13 wk (91 d) = 0.43 mg kg^{-1} d^{-1} for 13 wk; females were dosed daily (0.6 mg kg^{-1} d^{-1}) during gestation (21 d) = 0.6 mg kg^{-1} d^{-1} for 3 wk; and females were dosed at least 4 d/wk at 0.6 mg kg^{-1} d^{-1} during lactation (21 d) = 0.34 mg kg^{-1} d^{-1} for 3 wk. The time-weighted average (TWA) dose for this 133-d period is calculated as follows:

$$\text{TWA} = \frac{\Sigma[(0.43\ \text{mg/kg/d} \times 13\ \text{wk}) + (0.6\ \text{mg/kg/d} \times 3\ \text{wk}) + (0.34\ \text{mg/kg/d} \times 3\ \text{wk})]}{19\ \text{wk}}$$

$$\text{TWA} = 0.44\ \text{mg/kg/d}$$

This NOAEL is slightly lower than the NOAEL of 0.5 mg kg^{-1} d^{-1} from the Sasser et al. (1989c) study and is, therefore, being used as the basis for the RfD_c for lewisite. The selection of this NOAEL is supported by the available data set with the exception of the rabbit data, the validity of which is uncertain. The RfD_c for lewisite is calculated as follows:

$$RfD_c = \frac{NOAEL_{adj}}{UF_H \times UF_A \times UF_S \times UF_L \times UF_D \times MF}$$

where:

NOAEL = 0.6 mg kg^{-1} d^{-1}
$NOAEL_{adj}$ = 0.44 mg kg^{-1} d^{-1} (adjusted for discontinuous exposure and varying dosing protocol)
UF_H = 10 (sensitive subpopulations)
UF_A = 10 (interspecies extrapolation)
UF_S = 10 (extrapolation from subchronic to chronic exposure)
UF_L = 1 (NOAEL used)
UF_D = 3 (deficient data base)
MF = 1 (no additional modifying factor needed)

Therefore:

$$RfD_e = \frac{0.44 \text{ mg/kg/d}}{10 \times 10 \times 10 \times 3 \times 1}$$

$$RfD_e = 0.0001 \text{ mg lewisite/kg/d}$$

$$RfD_e = 0.1 \text{ µg lewisite/kg/d}$$

The derivation of an oral RfD_e for lewisite necessitated addressing several issues regarding the available data set: (1) interpretation of toxicity data from gavage/gastric intubation studies and (2) identification of the critical effect. The available data for lewisite toxicity are limited to gavage and gastric intubation administration studies. Although these routes of administration allow for more precise control of the administered dose as opposed to drinking water or feeding studies, in the case of lewisite (or any highly corrosive agent) this protocol imparts substantial caveats in data interpretation. First, the use of a sesame oil vehicle and the gavage/gastric intubation administration result in the gastric mucosae being exposed to a bolus of material in a vehicle that limits normal dispersion of the test article in the stomach. The decrease in dispersion causes an increase in contact time between the corrosive agent and a limited surface mucosal area, thereby increasing the potential for inflammatory responses observed in the described studies. Furthermore, the presence of an oil vehicle likely affects the physicochemical interactions at the chemical–tissue interface by altering the solubility and distribution of the chemical. Second, within the context of the RfD_e, intake of a corrosive agent as a bolus/oil suspension would not be toxicologically or physiologically analogous to exposure to the chemical agent via environmental media such as water. The critical effect of orally administered lewisite in animals appears to involve gastric lesions (rats and rabbits), possible developmental effects (rabbits), respiratory tract inflammation responses (rats and rabbits), and increased mortality (rats and rabbits). The increased mortality and respiratory tract inflammation responses reported in the available studies appear to be associated more with dosing errors or simple reflux of the corrosive, irritating lewisite. The reflux and subsequent respiratory tract response would be highly unlikely in an environmental exposure situation (i.e., drinking water contamination). Furthermore, the studies did not provide data affirming the respiratory responses to be a function of systemically mediated lewisite toxicity. Therefore, an RfD_e based upon available data is tenuous and difficult to verify.

The proposed RfD_e for lewisite underwent preliminary review (July 10–12, 1996) by the Material/Chemical Risk Assessment (MCRA) Working Group of the Environmental Risk Assessment Program (ERAP). The MCRA Working Group of ERAP represents multiagency (EPA, DOD, and DOE) input by individuals experienced in deriving and validating toxicity values. The MCRA Working Group agreed that the critical toxic effect observed in the lewisite studies (forestomach lesions) appears to be an artifact of administration and that the overall database for lewisite is not robust. Although it was recognized that

the structure of lewisite might imply toxic activity differing from inorganic arsenic, it was the consensus of the MCRA Working Group that the lewisite RfD be considered not verifiable because of the data deficiencies, and that the existing RfD for inorganic arsenic (3×10^{-4} mg kg^{-1} d^{-1}; USEPA 1990b) be used as a surrogate. This is considered a valid and justifiable approach inasmuch as the inorganic arsenic RfD and the proposed lewisite RfD are similar (3×10^{-4} vs. 1E-04 mg kg^{-1} d^{-1}, respectively) and that lewisite in environmental media is degraded to inorganic arsenic.

XIV. Agent CK (Cyanogen Chloride)

Toxicity data specific for agent CK (cyanogen chloride) are limited. The majority of available data for humans and animals concern the effects of short-term inhalation exposures; few experimental data addressing the toxic effects of long-term exposure to CK are available.

CK is considered an irritant as well as a systemic poison when absorbed into the bloodstream. The systemic effects of CK are dependent on its conversion to cyanide in the body. Aldridge and Evans (1946) reported that cyanide was detected in circulating blood of dogs immediately after inhalation of CK. An *in vitro* study with rat blood showed that CK is rapidly converted to cyanide by a reaction with hemoglobin and glutathione, which eventually liberates cyanide; however, the conversion of CK to cyanide was not complete (Aldridge 1951). In whole blood the conversion was about 30%, but was higher (to about 60%) in isolated red blood cells (Aldridge and Evans 1946). To estimate the conversion of CK to cyanide *in vivo*, the same investigators compared intravenous LD_{50} values for hydrogen cyanide (HCN) and CK in rabbits, assuming that the systemic toxicity of CK results entirely from the cyanide (CN^-) formed. Thus, the intravenous LD_{50} of 0.8 mg/kg for HCN and 2.5 mg/kg for CK amounts to a 75% conversion of CK to cyanide. The percent conversion was calculated by dividing 0.8 mg HCN/kg by 1.06 mg CK/kg [converted from 2.5 mg/kg using the molecular conversion factor of 26/61 (mol wt CK = 61; mol wt CN = 26)].

As presented in a review by the U.S. Air Force (USAF 1989), the cyanide ion is a quickly acting chemical asphyxiant that is readily absorbed from the alveolar membrane, intestinal mucosa, or skin, and rapidly appears in the blood. The more rapidly a critical concentration is attained in the tissues, the more severe the effects. At sufficient doses, cyanide produces rapid death. Signs and symptoms of acute cyanide exposure in humans include weakness, headache, confusion, nausea, vomiting, increased rate of respiration or slow, gasping respiration, and eye and skin irritation. At sufficient doses, these effects are followed by collapse, coma, and death. Minimum lethal doses of HCN for humans are approximately 50–90 mg by ingestion and approximately 100 mg/m^3 (duration of exposure not given) by inhalation. At sublethal doses, cyanide can be detoxified by normal metabolic pathways to the relatively nontoxic thiocyanate ion (USAF 1989). The detoxification process is efficient, preventing long-term bioaccumulation of cyanide (Ballantyne 1987). Thus, sublethal exposure to cya-

nide may lead to the development of signs of toxicity; as detoxification proceeds, these signs will disappear. However, massive doses will saturate the detoxification mechanisms, with an immediate onset of toxic effects and rapid death.

A. Toxicology

1. Acute Toxicity. Very limited data were available to assess the short-term toxicity of CK by the oral route of exposure. Sittig (1985) reported that ingestion of a lethal dose of CK causes dizziness, rapid respiration, vomiting, flushing, headache, drowsiness, drop in blood pressure, rapid pulse, unconsciousness, and convulsions, with death occurring within 4 hr. The estimated lethal dose for adults reported in the Hazardous Substances Data Bank ranges from 50 to 300 mg CK/kg (HSDB 1995). For rats and cats, the oral LD_{50} is 6 mg/kg (DA 1974; RTECS 1995d).

CK is also highly toxic by the inhalation, ocular, and dermal routes of exposure (Sax 1984; Weiss 1980). Although its toxic effects and mode of action are similar to those of HCN, CK is much more irritating, causing marked respiratory tract irritation with hemorrhagic exudates of the bronchi and trachea, and pulmonary edema (Hartung 1994). At similar exposure concentrations (300–400 mg/m^3), HCN and CK are rapidly fatal to man, but 50 mg/m^3 of HCN can be tolerated for about 30 min without immediate or late effects, whereas CK is an intolerable irritant at this concentration. Intermediate concentrations of CK may produce cyanide poisoning complicated by pulmonary edema (Hayes 1982). On the basis of extrapolation from animal data, the respiratory LCt_{50} in man has been estimated at about 11,000 mg-min/m^3 (WHO 1970). On this basis, CK is less than half as toxic as hydrogen cyanide, which has an estimated LCt_{50} of 5000 mg-min/m^3. The casualty-producing concentration (causing significant injuries or incapacitation) is estimated to be >7000 mg-min/m^3 for CK (WHO 1970).

Because of its irritating properties, CK vapors cannot be tolerated even at very low concentrations. Hartung (1994) reported that exposure to 2.5 mg/m^3 CK for 10 min is the lowest irritant concentration for humans; exposure to 5 mg/m^3 for 10 min or to 50 mg/m^3 for 1 min is intolerable; exposure to 120 mg/m^3 is fatal after 30 min; and exposure to 400 mg/m^3 is fatal after 10 min. One occupational study indicates that even lower concentrations (approximately 1.8 mg/m^3) of CK are unbearable, producing severe eye and nose irritation in exposed workers (ACGIH 1991).

CK has caused severe injuries to the human eye following exposure to 100 mg/m^3 for 2 min (Sax 1984). Exposure to this concentration produced immediate smarting of the eyes, with severe blepharospasm and lacrimation (Grant 1974). Liquid CK is also a severe skin irritant, causing second- and third-degree burns on short contact (Weiss 1980).

Acute toxicity data for experimental animals exposed to CK by inhalation are presented in Table 32. For the intravenous route of administration, LD_{50}

Table 32. Acute inhalation toxicity of agent CK in experimental animals.

Species	Exposure concentration (mg/m^3)[a]	Exposure time	Effect	Reference
Rat	1800	3 min	LC_{50}	NDRC (1946b)
Mouse	6000	30 sec	LC_{50}	NDRC (1946b)
	1000	3 min	Fatal	Flury and Zernik (1931)
Rabbit	800	7 min	LC_{50}	NDRC (1946b)
	3000	2 min	Fatal	Flury and Zernik (1931)
Guinea pig	2750	2 min	LC_{50}	NDRC (1946b)
Cat	6000	1 min	LC_{50}	NDRC (1946b)
	3000	3–3.5 min	Fatal	Flury and Zernik (1931)
	100	18 min	Fatal after 9 d	Flury and Zernik (1931)
Dog	3800	1 min	LC_{50}	NDRC (1946b)
	800	7.5 min	Fatal	Flury and Zernik (1931)
	120	6 hr	Fatal	Flury and Zernik (1931)
	50	20 min	Recovered	Flury and Zernik (1931)
Goat	2500	3 min	Fatal after 70 hr	Flury and Zernik (1931)
Monkey	4400	1 min	LC_{50}	NDRC (1946b)

[a]Values rounded to nearest whole integer.

values are 4 mg/kg for rats (NRC 1977), 2.5–3.2 mg/kg for rabbits (Aldridge and Evans 1946; DA 1974), and about 3 mg/kg for dogs and goats (DA 1974). Subcutaneous administration to rabbits, dogs, and pigeons yielded LD_{LO} values of 20, 5, and 9 mg/kg, respectively (RTECS 1995d).

The acute effects of CK in experimental animals appear to be similar to those observed in humans (Flury and Zernik 1931). At high concentrations (not specified), inhalation of CK induced effects that are generally associated with cyanide poisoning (paralysis, unconsciousness) as well as severe irritation of the respiratory tract, with hemorrhage and edema of the lungs.

Exposure to lower concentrations for longer exposure periods (specific data not provided) cause only respiratory irritation. In the same study, Flury and Zernik (1931) compared the time to appearance of acute toxic response following exposure to CK and HCN in several animal species. For example, exposure to 0.3 mg/L (300 mg/m^3) produced complete paralysis and unconsciousness in mice 210 and 105 sec after exposure, while 1 mg/L (1000 mg/m^3) produced the same effects 150 and 60 sec after exposure to CK and HCN, respectively.

2. Subchronic Toxicity. Reed (1920) recorded signs and symptoms of workers exposed to low concentrations of CK gas in a CK manufacturing plant. One of the workers who was exposed daily for 8 mon experienced three episodes of "severe" exposure to CK that resulted in dizziness, nausea, profuse lacrimation,

blurring of vision, gasping, coughing, staggering, and prostration that lasted for several hours. Persistent symptoms included muscular weakness, lassitude, lung congestion, skin irritation, hoarseness, conjunctivitis, edema of the eyelids, and burning urine. There were also periods of irregular pulse that bore no relation to the acute exposure episodes. During the 8-mon exposure period, the worker's weight decreased from 170 to 150 lb (77.1 to 68.0 kg), but increased to 160 lb (72.6 kg) 5 wk after cessation of exposure. Effects attributed to CK in other workers were chronic vomiting, diarrhea, frequent urination, persistent coughing, spasmodic pain in the respiratory muscles, cold perspiration, chronic dull headache, and weight loss.

No data were available concerning the subchronic toxicity of CK in animals. NTP (1993) conducted subchronic drinking water studies of sodium cyanide (NaCN) with rats and mice. Groups of F344/N rats and B6C3F$_1$ mice (10 per concentration/sex) were administered NaCN in drinking water at concentrations of 0, 3, 10, 30, 100, or 300 ppm for 13 wk. The average doses of NaCN (based on average water consumption values for wk 2–13 for all animals) were as follows: 0.3, 0.9, 2.7, 8.5, or 23.6 mg kg^{-1} d^{-1} (male rats); 0.3, 1.0, 3.2, 9.2, or 23.5 mg kg^{-1} d^{-1} (female rats); 0.5, 1.8, 5.1, 16.2, or 45.9 mg kg^{-1} d^{-1} (male mice); and 0.6, 2.1, 6.2, 19.1, or 54.6 mg kg^{-1} d^{-1} (female mice). Gross and histological examinations, sperm motility and vaginal cytology evaluations, and hematology, clinical chemistry, and urinalysis evaluations were performed on both species.

No deaths attributed to sodium cyanide administration occurred in either species. In animals exposed to 300 ppm, male rats had slightly lower (≤5%) final mean body weights and mean body weight gains than the respective controls. Water consumption by rats and mice in the 100 and 300 ppm groups was 10%–30% lower than that of controls; however, no clinical signs attributable to sodium cyanide or to dehydration were observed. Poor palatability was suggested as the cause of decreased water consumption. No gross or microscopic changes specifically related to cyanide toxicity occurred at any site in males or females of either species. In particular, no lesions were found in the brain or thyroid gland. Differences between absolute and relative organ weights (with the exception of reproductive tissue) of exposed and control animals were minor and sporadic and were not dose related; these differences were not considered to be related to sodium cyanide administration. Hematological, clinical chemistry, and urinalysis evaluations of rats and mice revealed minimal changes that were not considered biologically significant, although the decreased urine volume and increased urine specific gravity observed in male rats were consistent with the observed decreases in water consumption. Urinary thiocyanate (the primary metabolite of cyanide) increased with increasing exposure concentrations at all time points.

Kamalu (1993) evaluated the effects of linamarin, a cyanogenic glucoside, in a diet containing cassava (*Manihot esculenta* Crantz), fed to growing dogs for 14 wk. There were three groups of dogs (breed not given, but assumed to be beagles), each comprising six animals. One group was fed cassava that was

expected to release 10.8 mg HCN/kg cooked food, a second group was fed a control diet to which enough NaCN was added at feeding time to release 10.8 mg HCN/kg cooked food (to monitor the effects of HCN released from cassava), and a third group was fed a control rice diet containing no cyanide compounds. Each animal was given approximately 100 g diet/kg body weight. Although the body weight of dogs was not provided, based on a reference body weight of 12.7 kg, the estimated dose is 1.08 mg HCN kg^{-1} d^{-1}. The biochemical variables investigated included plasma electrolytes, serum proteins, plasma-free amino acids, plasma enzymes, and urine protein. Histopathological examination of liver, kidney, myocardium, testis, and adrenal gland was performed.

The cassava diet produced an increased plasma thiocyanate concentration, increased urinary protein excretion, decreased serum albumin, and decreased plasma K and Ca. The NaCN diet caused increased plasma thiocyanate and urinary thiocyanate excretion that was significantly ($p < .01$) higher than that of dogs fed cassava. However, urinary protein ($p < .01$) and serum albumin excretion were lower than that of dogs fed cassava, indicating that the amino acids were not utilized to the same extent as the control (rice diet) group. Neither experimental diet had an effect on plasma gamma-glutamyl transferase, alanine transferase, isocitrate dehydrogenase activities, and plasma Na, Mg, and P concentrations. The cassava diet caused generalized congestion, hemorrhage, and periportal vacuolation of the liver; swelling, vacuolation, and rupture of the epithelial cells of the proximal convoluted tubules of the kidney; myocardial degeneration; and adrenal gland degeneration. The NaCN diet caused nephrosis and adrenal gland hyperplasia and hypertrophy. Both experimental diets also produced testicular effects (see following). It was concluded that the observed changes that occurred when the cassava diet was consumed were not entirely caused by cyanide.

Hertting et al. (1960) treated three dogs orally with NaCN in a gelatin capsule for up to 14.5 mon. One dog received a daily dose of 0.27 mg CN^-/kg body weight for 13.5 mon; another dog received a daily dose of 0.53 mg CN^-/kg body weight for 16 wk and then a daily dose of 2.2 mg CN^-/kg body weight for 10.5 mon; and the third dog received a daily dose of 1.1 mg CN^-/kg body weight for 14.5 mon. One control dog was used. At daily doses of more than 0.53 mg CN^-/kg, there were signs of acute intoxication immediately after dosing; however, recovery occurred in less than 0.5 hr. In all treated dogs, histological examination revealed degenerative changes in ganglion cells of the CNS and especially in Purkinje cells of the cerebellum (necrosis, reduced RNA content, inflammation).

3. Chronic Toxicity. No data were available concerning the chronic toxicity of CK in humans or animals. Chronic oral cyanide toxicity in humans has been implicated in various nerve disorders observed among people living in certain regions of Africa where cassava is a dietary staple. Cassava contains cyanogenic glycosides that release cyanide when metabolized *in vivo* (USEPA 1984b; West-

ley 1980). However, the studies lack quantitative exposure information and fail to address complicating factors such as nutritional deficiencies or metabolic disorders that may also be involved. Neurological findings are generally correlated with increased thiocyanate levels in whole blood. Specific neuropathies (functional disturbances or pathological changes in the peripheral nervous system) observed included hyperreflexia (exaggeration of reflexes) of the upper limbs; spastic partial paralysis of the lower limbs; spastic dysarthria (imperfect articulation of speech); diminished visual acuity; changes in the cerebellum; and deafness (ATSDR 1995).

A major outbreak of more than 1000 cases of paraparesis (partial paresis affecting the lower limbs), affecting mostly women and children, was reported in Mozambique (Ministry of Health, Mozambique 1984a,b). A prolonged drought in the area had exhausted most food resources, except cassava, and because of the drought, the cyanide levels were particularly high in the cassava plants. Detoxification of the bitter varieties by sun-drying was inadequate because of general food shortage, and metabolic detoxification was probably reduced because of the absence of sulfur-containing amino acids in the diet. The raw and uncooked cassava (containing higher levels of cyanide than the cooked) was eaten mostly by women and children. The estimated daily cyanide intake was 15–31.5 mg (approximately 0.2–0.45 mg/kg) in selected affected families chosen for the study. The clinical findings ranged from headache, vomiting, and slight weakness to blindness and paralysis of all four limbs; frequently, acute signs and symptoms were seen 4–6 hr after ingestion of meals. The levels of intake may be compared with the estimated human lethal dose of 50–90 mg for HCN (USAF 1989) and could explain the frequent acute reactions after meals. Although these signs and symptoms could not be definitely attributed to chronic cyanide intoxication because both patients and controls had similarly high thiocyanate levels and there was no correlation to disease severity, the investigators indicated that the epidemiological features of the disease suggested that chronic cyanide intoxication caused by cassava ingestion played a major role in its etiology.

Chronic oral exposure of humans who use cassava roots as the main source of their diets has also been associated with thyroid effects. Ingestion of cassava, in combination with iodine deficiency, has been associated with a high incidence of goiter and cretinism in Zaire (USEPA 1985). Thyroid effects are generally attributed to thiocyanate, a metabolite that markedly inhibits the accumulation of iodine by the thyroid gland, thus decreasing the ability of the gland to maintain a concentration of iodine above that of the blood (ATSDR 1995).

Information derived from an occupational exposure study on hydrogen cyanide indicates that humans inhaling concentrations up to 10 ppm for 5–15 yr may develop subjective signs and symptoms such as headache, weakness, changes in taste and smell, throat irritation, vomiting, effort dyspnea, lacrimation, colic, precordial pain, and nervous instability (El Ghawabi et al. 1975). Also reported were mild to moderate thyroid enlargement and increased iodine

uptake by the thyroid. Long-term exposure to cyanides in the occupational setting has also been associated with dermatitis, severe nasal irritation, nervous disorders, and EKG abnormalities (Carmelo 1955; Hardy and Boylen 1983).

Two animal studies summarizing long-term oral exposure to cyanide are available. Howard and Hanzal (1955) maintained groups of 10 male and 10 female Carworth Farm rats for 104 wk on diets that had been fumigated with HCN at nominal concentrations of 100 and 300 mg HCN/kg diet. Because HCN volatilized from the food, fresh rations were prepared every other day. Results of the analysis of residues over the 2-yr duration of the study indicated an average drop in the dietary concentration of HCN during 2 d, from 100 mg/kg to 51.9 mg/kg for the low dose and from 300 to 80.1 mg/kg for the high dose. Thus, the average daily low and high concentrations were about 76 and 190 mg HCN/kg diet (73 and 183 mg CN^-/kg diet). From the data reported on food consumption and body weight, the average daily estimated doses of CN^- were 4.3 mg and 10.8 mg CN^-/kg body weight for low- and high-dose rats, respectively. The average food CN^- concentrations were estimated on the basis of the author's data for concentration at the beginning and at the end of each food preparation period and by assuming a first-order rate of loss of CN^- for the intervening period. Food consumption, growth rate, and survival of treated animals were comparable to those of controls. There were no gross signs of toxicity or any effects on organ-to-body weight ratios for liver, kidney, spleen, brain, heart, adrenals, or gonads. No histological lesions of the heart, lung, liver, spleen, stomach, intestines, kidneys, adrenals, testes, uterus, ovary, cerebrum, or cerebellum were seen in a "representative number" of animals examined. Elevated thiocyanate levels were noted in the plasma, liver, and kidney in both treatment groups at termination.

Philbrik et al. (1979) fed KCN in the diet to a group of 10 male rats (strain not specified) for 11.5 mon at a concentration of 1500 mg KCN/kg diet (600 mg CN^-/kg diet). Another group of 10 rats served as the control. Assuming that a rat in a long-term feeding study consumes a quantity of food equal to 5% of its body weight, the dietary level corresponds to a daily dose of about 30 mg CN^-/kg body weight. When compared with controls, the treated rats had a 40% reduction in mean body weight gain, 53% decrease in plasma thyroxine levels, and a 68% decrease in thyroxin excretion rates. There were no definite histopathological lesions in the thyroid, sciatic, optic, or other neural tissues. Mild degenerative changes in the myelin of the spinal cord were observed.

4. Developmental and Reproductive Effects. No data were available concerning the developmental and reproductive effects of CK in humans or animals. NTP (1993) performed reproductive tissue evaluations (epididymis and testes weights, sperm motility, sperm counts, and vaginal cytology) in F344/N rats and $B6C3F_1$ mice (10 per concentration/sex) administered 0, 30, 100, and 300 ppm NaCN in drinking water for 13 wk. The average doses of NaCN (based on average water consumption values for wk 2–13 for all animals) were as follows: 2.7, 8.5, or 23.6 mg kg^{-1} d^{-1} (male rats); 3.2, 9.2, or 23.5 mg kg^{-1} d^{-1} (female

Table 33. Effects of NaCN administered in drinking water for 13 wk on male F344/N rats.

Measurement	0 ppm	10 ppm (2.7 mg $kg^{-1}d^{-1}$)	100 ppm (8.5 mg $kg^{-1}d^{-1}$)	300 ppm (23.6 mg $kg^{-1}d^{-1}$)
Left epididymis weight (g)	0.448	0.437	0.425	0.417**
Left cauda epididymis weight (g)	0.162	0.150*	0.148*	0.141**
Left testis weight (g)	1.58	1.56	1.52	1.46**
Spermatid heads (10^7/testis)	17.86	16.94	16.58	15.42*
Spermatid count	89.28	84.68	82.90	77.10*
Sperm motility (%)	94.24	90.67*	92.09*	90.66*

*Significantly different ($p \leq .05$) from control group.
**Significantly different ($p \leq .01$) from control group.
Source: NTP (1993).

rats); 5.1, 16.2, or 45.9 mg kg^{-1} d^{-1} (male mice); and 6.2, 19.1, or 54.6 mg kg^{-1} d^{-1} (female mice). The results of the evaluations are summarized in Tables 33 and 34. In rats, left cauda epididymal weights were decreased in exposed males. The decreases were concentration dependent (93%, 91%, or 87% of control values at 30, 100, or 300 ppm, respectively) and statistically significant ($p \leq .05$ at 30 and 100 ppm; $p \leq .01$ at 300 ppm). At 300 ppm, the left epididymal and testis weights were 93% and 92% (both $p \leq .05$) of control values. Spermatid

Table 34. Effects of NaCN administered in drinking water for 13 wk on male $B6C3F_1$ mice.

Measurement	0 ppm	10 ppm (5.1 mg $kg^{-1}d^{-1}$)	100 ppm (16.2 mg $kg^{-1}d^{-1}$)	300 ppm (45.9 mg $kg^{-1}d^{-1}$)
Left epididymis weight (g)	0.049	0.047	0.047	0.044*
Left cauda epididymis weight (g)	0.017	0.016	0.015	0.014*
Left testis weight (g)	0.121	0.113	0.117	0.118
Spermatid heads (10^7/testis)	2.24	2.26	2.03	2.11
Spermatid count	69.94	70.80	63.28	66.06
Sperm motility (%)	92.38	90.63	91.43	89.52

*Significantly different ($p \leq .05$) from control group.
Source: NTP (1993).

heads/testis and spermatid counts in male rats at 300 ppm were significantly less ($p \leq .05$; 86%) than in the controls. Sperm motility in all groups of exposed males was lower ($p \leq .05$, 2%–4%) than that in controls, but these motility changes were not considered to be biologically significant. Female rats in the 100- and 300-ppm groups spent more time in proestrus and diestrus relative to estrus and metestrus than control females. Time spent in estrus was slightly reduced (from 35% to 24.2% at 300 ppm), thus reducing the time of receptiveness in the female, which may affect the reproduction rate. However, there were no experimental data to evaluate this possibility. Furthermore, the investigators indicated that the lack of a dose–response relationship suggests that the differences in female reproductive parameters are spurious and cannot unequivocally be attributed to sodium cyanide exposure.

In male mice at 300 ppm, the left epididymal and left cauda epididymal weights were 90% and 82% ($p \leq .05$), respectively, compared with those of controls. No changes in sperm motility or spermatid density occurred in male mice, and no significant changes in estrus cycle length were seen in female mice. The NTP investigators concluded that subchronic exposure to low doses of cyanide may produce mild but perhaps significant adverse effects on the male rodent reproductive system. They also indicated that the collective reproductive changes alone are probably insufficient to decrease fertility in rats; however, the interactive effects of fertilization and development were not evaluated. In addition, the relative sensitivity of humans to such changes is considered to be greater than that of rats (Working 1988). Therefore, the potential for adverse reproductive effects in human males following subchronic exposure to cyanide or cyanogenic compounds is thought to exist.

Several studies have been conducted with animals exposed to cyanogenic glycosides through diets containing cassava meal. However, as noted by Tewe and Maner (1981a), the adverse effects of inorganic cyanides may not be the same as those from organic cyanide sources such as cassava. When intact cyanogenic glycosides are ingested, probably little or no free HCN is released during the passage through the gastrointestinal tract, at least not before reaching bacteria in the lower intestine.

Singh (1981) administered rats a diet containing 50% or 80% cassava powder during the first 15 d of gestation. The 80% cassava diet caused increased resorptions and a low incidence of developmental abnormalities (microcephaly, open eyes, limb defects, and growth retardation). In the group consuming 50% cassava, the only finding was lower fetal body weights. Maternal weight gain was lower in both treated groups compared to controls from d 6 onward. The authors indicated that the observed effects could have been caused by the low protein content of the cassava diet. The doses of cyanide could not be determined from this study.

Frakes et al. (1986) exposed female Golden Syrian hamsters to cassava diets containing low and high levels of cyanide (approximately 0.6 and 7.9 mmol/kg, respectively). The diets were fed on d 3–14 of gestation. Body weight and food intake data were not provided; however, assuming a body weight of 0.14 kg for

hamsters and a food factor of 0.83 (fraction of body weight that is consumed per day as food), the estimated cyanide intake is 1.3 or 15.6 mg CN^- kg^{-1} d^{-1}, respectively, for the low- and high-cyanide cassava diet. No changes were observed in the number of implantations and resorptions, but low- and high-cyanide cassava-fed dams (both groups) gained significantly less weight than controls, and their offspring showed signs of fetotoxicity (reduced fetal body weight and reduced ossification). In addition, the high-cyanide diet was associated with a significantly increased number of runts compared with the control group.

In a two-generation study, Tewe and Maner (1981a) investigated the reproductive performance of 20 Wistar rats by adding KCN (500 mg CN^-/kg diet) to a cassava root flour-base diet that contained approximately 12 mg HCN/kg diet. Twenty control rats were fed the same basal diet. Assuming young growing rats and pregnant rats consume a food equivalent to 10% of their body weight, the basal diet provided about 1.2 mg CN^- kg^{-1} d^{-1}, and the CN^--supplemented diet provided about 51 mg CN^- kg^{-1} d^{-1}. The diets were administered starting about 20 d before gestation, during gestation, and through lactation and the postweaning period. No significant differences were found between treated animals and controls with respect to gestational weight gain, litter size, pup birth weight, food consumption, body weight change during lactation, weanling weights, and offspring mortalities. However, the offspring that were continued on the cyanide diets during the postweaning period consumed less food and grew at a significantly slower rate than the basal diet offspring, regardless of previous cyanide exposure (*in utero* and/or milk and/or diet). The protein efficiency ratio of rats exposed *in utero* and fed cyanide during the postweaning phase was significantly reduced compared with basal diet rats.

In a similar study, Tewe and Maner (1981b) evaluated the reproductive performance of pregnant Yorkshire pigs (6/group) administered cassava diets containing 0, 250, or 500 mg CN^-/kg fresh cassava beginning on the day after breeding and continued until parturition. According to the authors' calculations, the groups received a basal diet of low cyanide content (30 mg CN^-/kg diet) or the basal diet plus CN^-, which provided total CN^- levels of 277 or 521 mg CN^-/kg diet. The dose per day can be determined by multiplying the concentration of CN^- in the diet by 3.72 kg (amount of food given) to give a total dose per animal per day. The total dose was then divided by the estimated average body weight for the gestation period (218.6, 223.6, or 211.6 kg), providing an estimated intake of 0.51, 4.6, or 9.16 mg CN^- kg^{-1} d^{-1} for the basal diet and low- and high-cyanide groups, respectively. There were no observed effects on litter size, litter size at weaning, birth weight, and daily feed intake of sows and offspring or birth weights. Small increases in maternal thyroid weight were seen with increasing CN^- levels. At the high dose (521 mg CN^-/kg diet), pregnant sows had reduced thyroid activity, and significant increases of thiocyanate concentrations were seen in fetuses. Proliferative changes in the kidney glomeruli were seen in all three groups.

In a 14-wk study, Kamalu (1993) evaluated the effects of a cassava diet on spermatogenesis in growing male dogs. One group was fed cassava, which was

expected to release 10.8 mg HCN/kg cooked food; a second group was fed a control diet to which enough NaCN was added at feeding time to release 10.8 mg HCN/kg cooked food; and a third group was fed a control rice diet. The daily HCN intake was estimated at 1.08 mg kg^{-1} d^{-1}. Occasional abnormal germ cells in the seminiferous tubules and occasional sloughing of germ cells (but with remnants of Sertoli cells) were seen in the testes of animals fed the cassava diet. Spermatogenesis, however, appeared to be normal. The NaCN diet caused a significantly ($p < .01$) decreased relative frequency of testicular tubules containing spermatids in the lumen and marked testicular germ cell sloughing and degeneration. Therefore, the effect appears to be on developing spermatids in the seminiferous tubules.

When pregnant hamsters were administered NaCN by subcutaneously implanted osmotic minipumps that delivered cyanide at a rate of 6.125–6.517 mmol NaCN kg^{-1} hr^{-1} beginning on d 6 of gestation through delivery, severe malformations were observed at all doses greater than 6.125 mmol kg^{-1} hr^{-1}, and a dose of 6.517 mmol/kg caused 100% fetal mortality and some maternal deaths (Doherty et al. 1982). Malformations included exencephaly, encephalocele, nonclosure of the neural tube, and microphthalmia.

5. Carcinogenicity. No data were available concerning the carcinogenicity of CK in humans or animals. The currently available evidence gives no indication that cyanides are carcinogenic. Although no studies specifically analyzing for cancer have been conducted, a chronic dietary study of rats exposed to HCN at estimated doses of 10.8 mg CN^- kg^{-1} d^{-1} found no tumors in various tissues examined histologically (Howard and Hanzal 1955). The USEPA has assigned cyanide to Group D, not classifiable as to human carcinogenicity (USEPA 1996e). The International Agency for Research on Cancer has not adopted a cancer classification for CK or cyanides (IARC 1987c).

6. Genotoxicity. No genotoxicity studies were available for cyanogen chloride. Genotoxicity studies with cyanides have yielded mostly negative results. Cyanide in the form of sodium cyanide tested negative in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535 with and without S9 activation at concentrations up to 333 µg/plate (NTP 1993). Investigators performing other studies using as many as seven strains of *S. typhimurium* did not observe genotoxic effects with potassium cyanide (De Flora 1981; De Flora et al. 1984). Hydrogen cyanide at concentrations to 5 mg/plate was marginally mutagenic to *S. typhimurium* strain TA100 in the absence of metabolic activation, but not mutagenic to strain TA98 in the presence or absence of metabolic activation (Kushi et al. 1983). Negative responses were obtained in a *rec* assay in *Bacillus subtilis* (Karube et al. 1981) and in a DNA repair test in *Escherichia coli* WP67, CM871, and WP2 (De Flora et al. 1984). Sodium cyanide did not induce DNA strand beaks in cultured lymphoma cells (Garberg et al. 1988). In an *in vivo* study, a single oral dose of potassium cyanide that provided 1 mg cyanide/kg did not induce testicular DNA synthesis in mice (Friedman and Staub 1976).

B. Estimated Reference Dose

1. Selection of the Key Study. The most reliable data to use in calculating RfDs are subchronic or chronic human studies using the appropriate exposure route; however, such data for CK are limited to one subchronic occupational exposure study (Reed 1920) that did not provide exposure concentrations. There were no subchronic or chronic animal data from which to develop an RfD_e for CK. Acute human exposure data could be used to establish short-term exposure limits; however, there is insufficient information to indicate whether chronic adverse effects would be avoided if exposures were maintained below levels at which acute toxicity would occur.

The systemic toxicity of CK results from its transformation to free cyanide; thus, CK is expected to elicit the same toxic effects as cyanide. Therefore, in the absence of chemical-specific subchronic or chronic human or animal studies for CK, an oral RfD_e can be derived based on results of experimental studies with HCN or other cyanides. The nervous system, reproductive system, and thyroid are considered target organs for chronic toxicity of cyanides.

Studies considered most suitable for the derivation of an oral RfD_e are the subchronic drinking water study of sodium cyanide (NaCN) with rats and mice conducted by NTP (1993) and the epidemiological studies reported by the Ministry of Health, Mozambique (1984a,b). Both studies are considered co-critical studies for the derivation of the RfD_e for CK.

2. RfD_e Derivation. In the experimental study conducted by NTP (1993), groups of F344/N rats and $B6C3F_1$ mice (10 per concentration/sex) were administered five dose levels of NaCN (0, 3, 10, 30, 100, or 300 ppm) in drinking water for 13 wk. The average doses of NaCN were as follows: 0.3, 0.9, 2.7, 8.5, or 23.6 mg kg^{-1} d^{-1} (male rats); 0.3, 1.0, 3.2, 9.2, or 23.5 mg kg^{-1} d^{-1} (female rats); 0.5, 1.8, 5.1, 16.2, or 45.9 mg kg^{-1} d^{-1} (male mice); and 0.6, 2.1, 6.2, 19.1, or 54.6 mg kg^{-1} d^{-1} (female mice). Endpoints evaluated for all dose levels were histopathology, clinical chemistry, hematology, and urine chemistry; reproductive toxicity was evaluated at 30, 100, and 300 ppm. Concentrations of 100 ppm and greater resulted in reduced water consumption that was attributed to poor palatability. No clinically significant body weight, organ weight (except for reproductive tissues), histopathological, or clinical pathological changes were observed in either species. Compared to controls, oral ingestion of sodium cyanide caused alterations in reproductive parameters. In male rats, these alterations included dose-related decreased absolute cauda epididymis weights ($p \leq .05$ at 2.7 and 8.5 mg kg^{-1} d^{-1}; $p \leq .01$ at 23.6 mg kg^{-1} d^{-1}; decreased absolute epididymis and testis weights at 23.6 mg kg^{-1} d^{-1} ($p \leq .01$); and decreased numbers of spermatid heads/testis at 23.6 mg kg^{-1} d^{-1} ($p \leq .05$) (see Table 33). Sperm motility was marginally lower in all groups of male rats. Statistically significant ($p \leq .05$) decreased absolute left cauda epididymis weights were also seen at the lower doses (2.7 and 8.5 mg kg^{-1} d^{-1}). Effects on reproductive parameters in female rats (more time in proestrus and diestrus relative to estrus and metestrus

than control females) at 9.2 and 23.5 mg kg^{-1} d^{-1} could not be unequivocally attributed to cyanide treatment. In male mice at 45.9 mg kg^{-1} d^{-1}, left epididymal and cauda epididymal weights were lower ($p \leq .05$) compared with those of controls. No changes in sperm motility or spermatid density occurred in male mice, and no significant change in estrus cycle length was seen in female mice.

Based on the reproductive changes observed in male rats (decreased epididymis, cauda epididymis, and testis weights and decreased spermatid counts), 23.6 mg NaCN kg^{-1} d^{-1} is identified as the LOAEL and 8.5 mg NaCN kg^{-1} d^{-1} is identified as the NOAEL. The decreased epididymis, cauda epididymis, and testis weights observed in conjunction with decreased spermatid heads and spermatid counts at 23.6 mg NaCN kg^{-1} d^{-1} implies a physiological change in rats that is of biological significance (decreases in sperm motility were not considered to be biologically significant). In the low- and mid-dose groups, the only statistically significant change was a decrease in left caudal epididymis weight. In our scientific judgment, this organ weight change in the absence of changes in spermatid counts is not considered to be an adverse effect. Using the molecular weight conversion factor of 26/49 (mol wt NaCN = 49; mol wt CN = 26), the LOAEL and NOAEL for CN^- are 12.5 and 4.5 mg kg^{-1} d^{-1}, respectively.

Studies by Aldridge and Evans (1946) indicated that CK may not release all the available cyanide in the body; they suggest using a 75% conversion factor (based on a comparison of intravenous LD_{50} values for HCN and CK in rabbits). However, there are no supporting studies to justify the use of a 75% conversion factor for the derivation of an RfD_c. Therefore, the RfD_c for CK assumes 100% conversion and is based on a maximum number of molar equivalents (1) of CN^- being released. The calculation follows:

$$RfD_c = \frac{NOAEL}{UF_H \times UF_A \times UF_S \times UF_L \times UF_D}$$

where:

NOAEL = 10.6 mg kg^{-1} d^{-1} [no-observed-adverse-effect level; converted from 4.5 mg $kg^{-1}d^{-1}$ for CN^- using the molecular weight conversion factor of 61/26 (mol wt CK = 61; mol wt CN = 26)]

UF_H = 10 (to protect sensitive human subpopulations)

UF_A = 10 (animal to human extrapolation)

UF_S = 3 (subchronic to chronic exposure extrapolation; full factor of 10 not used because a chronic oral study is available)

UF_L = 1 (NOAEL is used)

UF_D = 1 (data base for cyanide considered to be adequate)

Therefore:

$$RfD_c = \frac{10.6 \text{ mg/kg/d}}{10 \times 10 \times 3 \times 1 \times 1}$$

$$RfD_c = 0.035 \text{ mg CK/kg/d}$$

Although precise exposure data are lacking, an oral RfD_c can be also estimated from the human data reported by the Ministry of Health, Mozambique (1984a,b). A major outbreak of more than 1000 cases of partial paresis of the lower limbs occurred in Mozambique, affecting mostly women and children. The investigators estimated that the daily cyanide intake derived from cooked and raw cassava was 15–31.5 mg (approximately 0.2–0.45 mg CN^- kg^{-1} d^{-1}, based on a 70-kg body weight) in selected families chosen for the study. The average intake of 0.33 mg kg^{-1} d^{-1} can be considered a LOAEL for cyanide, with nervous system toxicity as the critical effect. A NOAEL was not identified. Using a molecular conversion factor of 61/26 (mol wt CK = 61; mol wt CN = 26), the LOAEL for CK is 0.77 mg kg^{-1} d^{-1}, and the RfD_c is derived as follows:

$$RfD_c = \frac{\text{LOAEL}}{UF_H \times UF_S \times UF_L \times UF_D}$$

where:

LOAEL = 0.77 mg CK/kg/d
UF_H = 1 (sensitive individuals)
UF_L = 10 (LOAEL-to-NOAEL extrapolation)
UF_S = 3 (subchronic-to-chronic extrapolation)
UF_D = 1 (data base)

Therefore:

$$RfD_c = \frac{0.77 \text{ mg/kg/d}}{1 \times 10 \times 3 \times 1}$$

$$RfD_c = 0.026 \text{ mg CK/kg/d}$$

A total uncertainty factor of 30 was applied. A UF_H of 1 was applied because the subject population of starving woman and children who had consumed cassava is considered to be a sensitive subpopulation. A standard default value of 10 was used for extrapolating from a LOAEL to NOAEL. Although the affected population was chronically exposed to cassava in their diet, a UF_S of 3 was applied because the maximum intake occurred during a short drought period. The data base for cyanide is considered to be adequate for deriving an RfD_c for cyanogen chloride.

Considering the epidemiological study (Ministry of Health, Mozambique 1984a,b) and the drinking water study with rats (NTP 1993) as co-critical studies, the RfD_c for CK is 0.03 mg kg^{-1} d^{-1}. The critical effects identified in the two studies (nervous system and reproductive toxicity, respectively) are consistent with toxic effects of cyanides.

3. Confidence in the RfD_c.

Studies: Medium to high
Data base: Medium
RfD: Medium to high

Confidence in the experimental drinking water study by NTP (1993) is high. It was well designed with respect to exposure protocol, number of animals, and exposure duration, and identified a LOAEL and NOAEL. The confidence in the epidemiological study (Ministry of Health, Mozambique 1984a,b) is medium, primarily because of uncertainties associated with cyanide consumption from cassava. Although the data base for CK is inadequate, the data base for cyanide contains subchronic and chronic bioassays in more than one species and reproductive/developmental toxicity studies in more than one species. Confidence in the data base for cyanide is considered medium. Medium to high confidence in the RfD_e follows.

4. Supporting Studies. The oral RfD_e of 0.03 mg kg^{-1} d^{-1} for CK derived from human (Ministry of Health, Mozambique (1984a,b) and animal data (NTP 1993) are supported by an earlier RfD of 0.05 mg kg^{-1} d^{-1} derived by EPA (USEPA 1996d), based on the results of the chronic dietary study of rats exposed to HCN conducted by Howard and Hanzal (1955). In this study, male and female Carworth Farms rats were administered a diet fumigated with HCN at nominal concentrations of 100 or 300 ppm for 104 wk. From the data reported on food consumption, body weight, and estimated CN^- content of food, the daily doses were 4.3 and 10.8 mg CN^- kg^{-1} d^{-1} for the low- and high-dose rats, respectively. There were no treatment-related effects on growth rate and no gross signs of toxicity. No histopathological lesions were seen in various tissues examined, including brain and reproductive organs. Because there were no observed adverse effects, the study provided a NOAEL (10.8 mg CN^- kg^{-1} d^{-1}) but not a LOAEL. Using a molecular conversion factor of 61/26 (mol wt CK = 61; mol wt CN^- = 26), the NOAEL for CK is 25.3 mg kg^{-1} d^{-1}. Applying an UF factor of 100 (10 for species extrapolation and 10 for sensitive populations) and a modifying factor (MF) of 5 (to account for the apparent tolerance to cyanide when it is ingested with food rather than when it is administered in drinking water), the resulting RfD for oral exposure to CK is 0.05 mg kg^{-1} d^{-1}.

In a study conducted by Philbrick et al. (1979), weight loss, thyroid effects, and myelin degeneration were observed in rats maintained for 11.5 mon on a diet containing KCN (equivalent to approximately 30 mg CN^- kg^{-1} d^{-1}). Because only one dose level was tested, a NOAEL could not be identified from this study.

Testicular germ cell sloughing and degeneration and a decreased number of testicular tubules containing spermatids were observed in growing male dogs fed a NaCN-supplemented diet that was expected to release 10.8 mg HCN/kg food (Kamalu 1993). Based on a daily food intake of 100 g food/kg body weight (value provided by author) and a reference body weight of 12.7 kg, the estimated daily dose is 1.08 mg HCN/kg (equivalent to 2.5 mg CK/kg). The LOAEL for CK derived from this study is 2.5 mg CK kg^{-1} d^{-1}, with reproductive toxicity as critical effect. This study was not selected for derivation of the RfD_e because the dosing protocol was not adequately described by Kamalu (1993).

XV. Environmental Fate and Effects

For all the environmental fate discussions in this review, data for environmentally realistic conditions are used unless otherwise specified. The pH of most natural waters ranges between 6 and 9 (Howells 1983), with most freshwaters of the U.S. at a pH of 7, temperature of 20 °C, and 0.00 ionic strength in non-winter months (Mabey and Mill 1978). These factors influence the hydrolysis of chemical agents. Soils in general have a relative humidity >98%, and hydrolysis and oxidation reactions are likely to occur (Morrill et al. 1985), making transport of the agents to groundwater unlikely. Hydrolysis reactions, biodegradation, soil sorption, and the slow rate of flow through the unsaturated soil zone limit the amount of any of the agents reaching subsurface aquifers. Under high desert or semiarid conditions, hydrolysis would be a less likely fate mechanism, but at the same time, transport to groundwater via leaching would also be unlikely. Under conditions of low soil humidity, volatilization from soil would become a more important mechanism of removal.

Compounds are potentially subject to photodegradation if they absorb light in the visible-UV light region of 290–750 nm (Lyman et al. 1982). Photolysis may take place in the atmosphere, in surface waters, and on soil. With the exception of lewisite, light absorption occurs at less than 290 nm, indicating little photodegradation for the majority of the agents. Physical and chemical properties of chemical warfare agents used in the following discussions may be found in Section II and in Tables 4–6.

Sulfur mustard can be considered environmentally persistent because it is chemically stable and of low volatility. When protected from weathering conditions, it may persist in soil for years. VX is moderately persistent because of low volatility and slow rate of hydrolysis. The G-agents can be considered non-persistent on the basis of volatility and hydrolysis rates. Depending on environmental conditions, their half-lives may be measured in hours to days. Lewisite is rapidly hydrolyzed but the insoluble oxide formed is stable in the environment. In addition, arsenical degradation products of lewisite persist in the environment. Because of its extreme volatility and relatively rapid hydrolysis, cyanogen chloride is not persistent in the environment.

Although two of the nitrogen mustards are not specifically treated in this review, their chemical properties resemble those of sulfur mustard and their fate in the environment is briefly discussed. Based on chemical and physical properties, HN3 [$(CH_2CH_2Cl)_3N$] can be considered environmentally persistent and HN1 [$(CH_2CH_2Cl)_2NC_2H_5$) and HN2 [$(CH_2CH_2Cl)_2NCH_3$] can be considered moderately persistent.

A. Mustard Agents

1. Environmental Fate. There are few published data on the fate of mustard agents in air, water, or soil; most of the available data, including chemical and

physical properties, indicate environmental persistence, particularly for sulfur mustard.

Air. Although the vapor pressure of distilled sulfur mustard is low (0.115 mmHg at 25 °C), it is sufficient for mustard to be in the air immediately surrounding droplets of the liquid. The principal UV absorption band of HD in cyclohexane at 205 nm (Rewick et al. 1986) suggests that direct photolysis in air is not likely to be a significant mode of degradation (HSDB 1997). Atkinson (1987) reported on the estimated rate constants of reactions of OH radicals with organic compounds. Using the data of Atkinson (1987), Syracuse Research Corporation calculated that in the presence of $5 \times 10^5/cm^3$ photochemically produced hydroxyl radicals in the atmosphere (assumed average concentration in nonsmog conditions), HD will react with an estimated rate constant of 11.4×10^{-12} cm^3/mol-sec at 25 °C or a half-life of 1.4 d (HSDB 1997).

The volatility of HN3 is low, about 100 mg/m^3 at 20 °C, and a toxic concentration would not develop in the atmosphere at contaminated sites. The volatilities of HN1 and HN2 are greater than that of HN3, approximately 2000 and 3500 mg/m^3 at 25 °C, respectively (Franke 1982).

Water. The hydrolysis of chemical agents in water is directly related to their solubility; thus, water solubility greatly influences their persistence. All the mustard agents have limited solubility in water at neutral pH. Because of the low water solubility of H and HD and virtual insolubility of HT, bulk amounts of mustard agents persist undispersed under water for some time. Although HD has been reported to hydrolyze in distilled water with a half-life of 8.5 min at 25 °C, hydrolysis is limited by the very slow rate of solution and the hydrolysis rate is essentially the rate of solution. Low temperatures decrease the rate of hydrolysis and result in greater persistence.

Hydrolysis of HD is surface controlled, with products formed at the HD–water interface and then diffusing into the bulk water phase (MacNaughton and Brewer 1994; Rosenblatt et al. 1975, 1995; Small 1984; Yang et al. 1992). The hydrolysis mechanism is complex and occurs by two routes, both of which lead to formation of thiodiglycol and hydrochloric acid (Fig. 2). In a dilute aqueous solution, dissolved HD is rapidly converted first to a sulfonium ion and then to the hemi-mustard and thiodiglycol; in the presence of insufficient water to initially dissolve all available HD, several sulfonium ion aggregates (thiodiglycol-mustard aggregates) are formed at the water–HD interface. The sulfonium ion aggregates are stable products at ambient temperatures (Yang et al. 1992) and would shield the bulk of the material from further dissolution (Small 1984). Thus, the formation of aggregates probably contributes to environmental persistence. As shown in Fig. 2, hydrolysis of the hemi-mustard-thiodiglycol aggregate releases thiodiglycol. Chemical formulas and Chemical Abstract Service numbers of the major hydrolysis products are listed in Table 35.

The laboratory studies reviewed here, however, used unbuffered waters in which the pH decreased throughout the study because of the release of chlorine

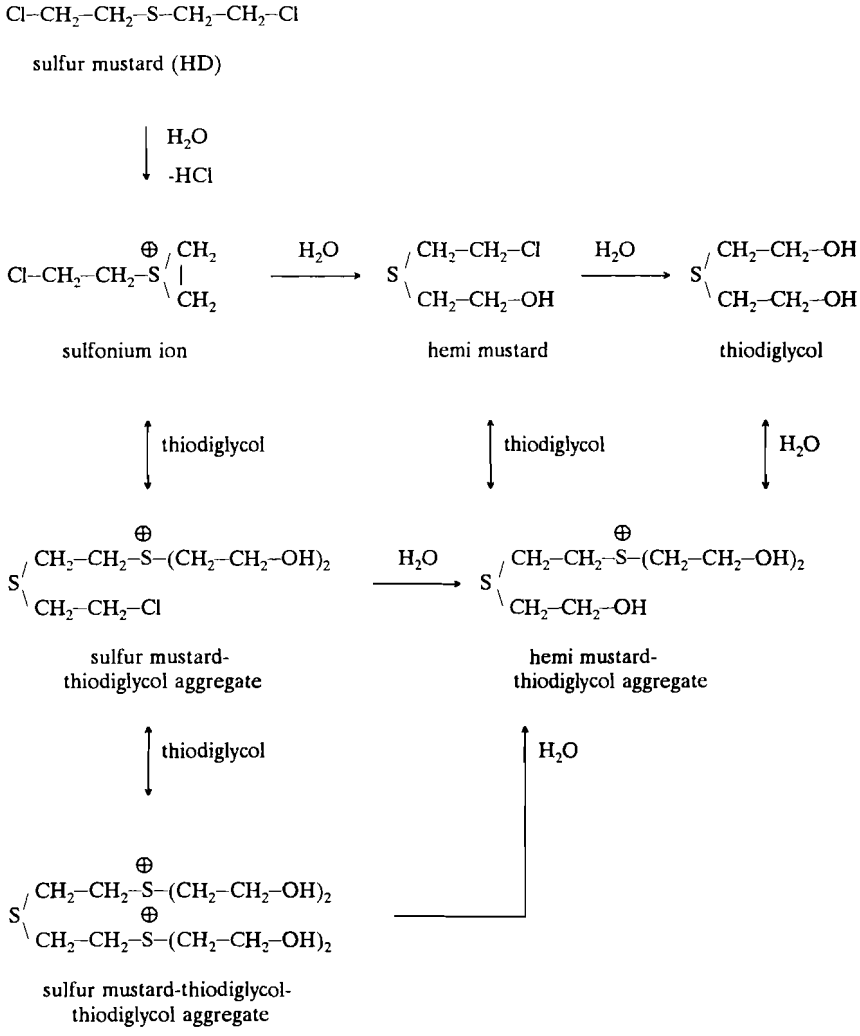


Fig. 2. Primary hydrolysis pathways of agent HD in the environment.

and subsequent formation of hydrochloric acid. In reviewing these and additional studies, Small (1984) pointed out that, at the pH range encountered in natural waters which would also be buffered, the hydrolysis rate would not be strongly pH dependent. In addition, conditions required to produce reversible hydrolysis are not normally encountered in the environment. In the presence of oxidants such as chlorine or hydrogen peroxide in the water, sulfur mustard readily undergoes oxidation to mustard sulfoxide and mustard sulfone. In

Table 35. Agent HD hydrolysis products.

Product	Formula	CAS No.
bis(2-Hydroxyethyl)-2-(2-chloroethylthio) ethyl sulfonium chloride (sulfur mustard-thiodiglycol aggregate)	$C_8H_{18}ClO_2S_2{\cdot}Cl$	64036-91-5
bis-2(bis(2-Hydroxyethyl)-sulfonium ethyl) sulfide dichloride (sulfur mustard-thiodiglycol-thiodiglycol aggregate)	$C_{12}H_{28}O_4S_3{\cdot}2Cl$	64036-79-9
bis(2-Hydroxyethyl)-2-(2-hydroxyethylthio) ethyl sulfonium chloride (hemi-mustard-thiodiglycol aggregate)	$C_8H_{19}S_2O_3{\cdot}Cl$	64036-92-6
Hydrochloric acid (in acidic solution)	HCl	7647-01-0
Thiodiglycol	$C_4H_{10}O_2S$	111-48-8

Sources: DA (1974, 1988); MacNaughton and Brewer (1994); Sanches et al. (1993); Small (1984).

weakly alkaline solution, the sulfone is dehydrochlorinated to divinyl sulfone (Rosenblatt et al. 1975).

According to Rosenblatt et al. (1975), mustard agent will not travel through groundwater in solution because of its low solubility and rapid hydrolysis when dissolved. Thus, HD is not normally found in groundwater. The hydrolysis product thiodiglycol is miscible with water (Budavari et al. 1989), and may be found in surface water or may leach to groundwater.

A Henry's law constant of 2.4×10^{-5} atm-m^3/mole (Small 1984) indicates that volatilization from water could be significant. However, in the absence of turbulence and at low temperatures, large quantities of HD would persist under water for considerable periods and retain blister-forming properties (Sanches et al. 1993).

HD spilled into seawater would probably sink (specific gravity, 1.27 at 20 °C), remaining on the bottom where it would slowly dissolve, resulting in no more than a few ppm of unhydrolyzed mustard in the supernatant water (Epstein et al. 1973). Some might form a surface film on the water where it would be removed within a few days by hydrolysis and volatilization (Epstein et al. 1973; Madema 1986). However, high levels of chlorine in the water inhibit hydrolysis, and therefore hydrolysis in seawater is slower than in freshwater (Epstein et al. 1973).

Little information on the hydrolysis of nitrogen mustards was located. Nitrogen mustards are stable during storage, and, in the presence of water, form dimers (Forsman et al. 1979; Franke 1982). Hydrolysis of HN3 is slower than that of the sulfur mustards, but the hydrolysis of HN1 and HN2 is probably more rapid. The mechanism of hydrolysis is similar, with formation of a cyclic intermediate. Solubilities range from 0.16 g/L for HN3 to 12 g/L for HN2

(Franke 1982). For HN1, solubility is expected to increase with decreasing temperature, and a half-life of 12.5 d at 5 °C for HN1 and all its toxic hydrolysis products, including the intermediate chlorohydrin, has been calculated (Epstein et al. 1973). The rate of hydrolysis in freshwater and seawater is expected to be similar. Epstein et al. (1973) identified the final hydrolysis product of HN1 as bis(β-hydroxyethyl)amine. Franke (1982) identified *N*-methyl-2-hydroxy-2-chlorodiethyl ammonium chloride, *N*-methyl-bis-(2-hydroxyethyl) ammonium chloride, and dimerization products as hydrolysis products of HN2 under laboratory conditions. The hydrolysis of HN3 is slower and complicated by the formation of reactive intermediates and dimerization products. In a 1% aqueous solution, bis(2-chloroethyl)-2-hydroxyethyl ammonium chloride and 2-chloroethyl-bis-(2-hydroxyethyl) ammonium chloride were identified after 20 and 72 hr, respectively.

Soil. Sulfur mustard is lost from the surface of soil primarily by evaporation, whereas mustard buried deep in the soil where it cannot vaporize or undergo weathering has been known to remain undecomposed for years (Small 1984). Studies of the persistence of sulfur mustard on surfaces, including soil, were reviewed by Small (1984) and Watson and Griffin (1992). Volatilization from soil was related to temperature, wind speed, and soil type. Droplets deposited on surfaces would evaporate slowly whereas bulk quantities would remain where initially deposited during cool weather or under winter/arctic conditions. Predicted persistence times for drops applied to soil (nominal surface density of 50 g/m^2) under various conditions of wind and rain were 1122–2215 hr at 0 °C and 30.5–51.2 hr at 25 °C (Puzderliski 1980). Several studies reviewed by Small (1984) and Watson and Griffin (1992) indicated persistence for weeks to decades in military testing areas and land dumps.

Another reason for the persistence of sulfur mustard is its characteristic freezing at moderate temperatures (13 °–15 °C) (Small 1984). Studies of the persistence of sulfur mustard performed at low temperatures (−1 °C) under actual field conditions in Norway show that small solid particles are formed on the snow surface. The droplets disappeared fairly rapidly, however, primarily by evaporation, and after 2 wk only 0.0001% remained (Johnsen and Blanch 1984; NMFA 1982, 1983).

Under conditions of low relative humidity (27%–35%) and ambient temperature (21 °–25.5 °C), 7%–32% of mustard experimentally applied to soils was recovered in the first 6 hr and 12%–66% was recovered by the time no more H vaporized (15–55 hr) (Epstein et al. 1973). The rate of vapor generation and the recovery depended on soil pH, moisture content, and chemical and physical characteristics of the soils.

Theoretically, HD can be biodegraded in soil via the thioether oxidation pathway forming bis-(2-chloroethyl)-sulfoxide and the corresponding sulfone, which are both water insoluble and highly toxic (Morrill et al. 1985). Mustard can also be biodegraded via reductive dehalogenation and dehydrohalogenation, although these pathways would be very slow. Removal of the chlorine atom results in a

nontoxic metabolite. Although the foregoing biodegradation pathways are suggested, biodegradation of mustard has not been achieved under laboratory conditions, probably because it is toxic to microorganisms.

Two common degradation products of HD that persist in the environment are 1,4-oxathiane and 1,4-dithiane. 1,4-Oxathiane is formed by dehydrohalogenation of partially hydrolyzed mustard while 1,4-dithiane is a thermal degradation product of mustard formed by dechlorination. Both products are groundwater contaminants in the Rocky Mountain Arsenal area (Sanches et al. 1993).

In light of the selection by the Army Chemical Stockpile Disposal Program of chemical neutralization (hot water hydrolysis followed by biodegradation of the hydrolysate) as an alternative technology to incineration for chemical demilitarization of HD (PMCD Newsletter 1997), the biodegradation of hydrolysis products is relevant. In laboratory studies that tested various conditions of temperature, HD concentration, and NaOH concentration, HD was completely hydrolyzed to thiodiglycol as well as ethers and thioethers (Beaudry et al. 1995). Two strains of the bacterial species *Pseudomonas pickettii* and *Alcaligenes xylosoxidans* (ssp. *xylosoxidans* strain SH42) were isolated from areas purported to have been previously contaminated with HD. These strains were capable of utilizing thiodiglycol as their sole source of carbon for growth (Beaudry et al. 1995; Harvey and DeFrank 1993). When mustard was hydrolyzed before inoculation with the bacteria, up to 97% of the carbon-containing hydrolysis products were degraded. Two different microtoxicological tests detected no toxicity in the resulting medium. Lee et al. (1997) reported that thiodiglycol was completely degraded by *A. xylosoxidans* strain SH91 in laboratory-scale stirred-tank reactors.

No data on the biodegradation of nitrogen mustards were located. Nitrogen mustards can theoretically be biodegraded via reductive dehalogenation and dehydrohalogenation mechanisms, but these processes would be very slow. HN1 and HN2 can be degraded via oxidation dealkylation (N-dealkylation for HN1 and C-dealkylation for HN2); the metabolites would possess vesicant properties (Morrill et al. 1985).

2. Ecotoxicology. A series of experiments on the toxicity of mustard agent (H) and several degradation products to aquatic organisms were performed by the National Defense Research Committee of the Office of Scientific Research and Development (Buswell et al. 1944). In these experiments, the toxicity of mustard was related to the rate at which mustard went into solution. Mustard added to fish aquaria (25 °C) formed globules in the bottom of the tanks, and amounts equivalent to 25–50 ppm were required for lethality for fish.

Therefore, the mustard was dissolved in alcohol or dioxane, and the following results are based on mustard in solution at the beginning of static tests. Mortalities of bluegill sunfish (*Lepomis macrochirus*) at 30 d in control and treated tanks (0, 1, 2, and 5 mg/L) were 0/30, 0/30, 22/30, and 30/30, respectively. The threshold for lethality was given as 2 mg/L. Similar results were observed with red-eared sunfish (*Lepomis microlophus*) and black bullheads

(*Ameiurus melas*); largemouth bass (*Micropterus salmoides*) were less sensitive, with a threshold for lethality above 5 mg/L. At a concentration of 1000 mg/L, thiodiglycol was not toxic to small bluegills within a 42-d observation period. The sulfur mustard-thiodiglycol-thiodiglycol aggregate was lethal to bluegills at 1000 mg/L or higher in 30-d tests.

The toxicity of H to a variety of phytoplankton and aquatic plants (water milfoil, parrot's feather, and water crowfoot) was tested under static conditions at concentrations of 5 and 50 mg/L (Buswell et al. 1944). H had no effect other than a delay of phytoplankton succession by 2–3 d and an initial wilting followed by revival of the higher plants.

Treatment with mustard agent may kill or injure terrestrial plants and decrease germination of seeds. Liquid sulfur mustard is phytotoxic, producing leaf necrosis in several species of terrestrial plants (Fichet 1942). Hassett (1963) reported observations of defoliation of trees and the loss of ground cover following the use of H in World War I. Perera and Thomas (1986) reported on contaminated soils and foliage and barren areas resulting from the manufacture and disposal of mustard agent in England during and after World War II. Mustard taken up by plants probably undergoes hydrolysis (Rosenblatt et al. 1975). The metabolite, thiodiglycol, applied by aerial application at 1 lb/acre, had no effects on several crop species including beans, oats, rice, soybeans, and radishes (Rosenblatt et al. 1975).

Rosenblatt et al. (1975) reviewed the toxicity of H to microorganisms. The studies addressed inactivation as well as mechanisms of action and mutagenicity but provided little data on actual toxic concentrations. Bacteria, phages, viruses, and yeasts were inactivated at similar concentrations, which were lower than concentrations that inactivated some enzyme systems. As noted previously, the toxicity of H to microorganisms probably precludes biodegradation.

The nitrogen mustards are less toxic to aquatic organisms than sulfur mustard. Thresholds for lethality (30-d) for the black bullhead were HN1, 25 mg/L; HN2, 10 mg/L; and HN3, 8 mg/L (Buswell et al. 1944). If introduction of the fish into the tanks containing HN3 at 25 mg/L was delayed for 12 hr, all fish survived, indicating a loss of toxicity, presumably through hydrolysis. The nitrogen mustards were less toxic than sulfur mustard to phytoplankton and higher aquatic plants (Buswell et al. 1944). The saturated vapor of nitrogen mustard (HN2) applied to the pollen of corn (*Zea mays*) for 2 min or more caused death or sterility of the fertilized seeds (Gibson et al. 1950).

B. Nerve Agents

1. Environmental Fate. The primary degradation mechanism for nerve agents in the environment is hydrolysis to the corresponding alkyl methylphosphonate, followed, for most of the agents, by slow hydrolysis to methyl phosphonic acid. The alkyl methylphosphonates may persist for years in the environment. Degradation or decomposition in soil may be faster than in water as a result of the variety of available processes and catalysts.

Air. G-agents are more volatile than V-agents. Because of their high volatility, the G-agents are expected to rapidly disperse, whereas the evaporation rate of VX is so small that a spill is not expected to result in significant atmospheric dispersion (DA 1988). When used as a chemical agent in aerial sprays or munitions, VX droplets are subject to gravitational settling (Sage and Howard 1989).

Although no data on the fate of nerve agents in the atmosphere were located, a VX simulant ($[CH_3CH_2O][CH_3CH_2S]P[S][CH_3]$) and chemically related pesticides appear to be resistant to photodegradation (Clark 1989). Because VX does not absorb UV radiation above 290 nm (Rewick et al. 1986), photodegradation does not appear to be a significant degradative process. However, according to Kingery and Allen (1995) the nerve agents and their degradation products can be degraded efficiently by photolysis and/or radical oxidation, although there is a paucity of rate information applicable to environmental fate. Kingery and Allen (1995) reported a study that measured direct photolysis of GD. The source of irradiation was a mercury lamp, and percent degradation was compared to nonirradiated samples. After 2 hr, GD degraded under both dry and wet conditions; the amount degraded to pinacolyl methylphosphonic acid was 10% in dry air (both with and without irradiation) and, in wet air, 22%–27% without irradiation and 47%–56% with irradiation. The authors concluded that some increase in the decomposition rate of nerve agents is caused by photolysis and that the rate depends on latitude and season. Based on structure–activity relationships, VX is predicted to react with photochemically produced hydroxyl radicals in the troposphere with an estimated half-life of 0.24 d (Atkinson 1987).

Water. Hydrolysis is dependent on water solubility. VX is less soluble in water than the G-agents and is relatively resistant to hydrolysis (Franke 1982). Reported half-lives in water at 25 °C and pH 7 range from 400 to 1000 hr (Clark 1989). Hydrolysis produces a variety of degradation products. As indicated in Fig. 3, hydrolysis of VX involves three pH-delineated regions where different products are formed (Clark 1989; Epstein et al. 1974; Kingery and Allen 1995; MacNaughton and Brewer 1994). At pH values less than 7 or greater than 10, the primary pathway is cleavage of the P–S bond to yield ethyl methyl phosphonic acid and diisopropylethyl mercaptoamine. Under alkaline conditions (pH > 10), the latter compound can be oxidized to bis(2-diisopropylaminoethyl) disulfide or react with ethyleneimmonium ion to form bis(2-diisopropylaminoethyl) sulfide. At pH values of 7–10, this pathway competes with dealkylation of the ethoxy group (cleavage of the C–O bond), the latter pathway yielding *S*-(2-diisopropylaminoethyl) methylphosphonothioate (also called EA 2192) and ethanol. Methyl phosphonic acid is slowly formed by hydrolysis of ethyl methylphosphonic acid (Kingery and Allen 1995) and has been isolated from VX-contaminated soil (Small 1984). The sulfur-containing products such as *S*-(2-diisopropylaminoethyl) methylphosphonothioate are toxic and relatively stable in water. Cleavage of the S–C bond may also occur, forming ethyl methylphosphonothioic acid, diisopropylaminoethanol, and ethyleneimmonium ion (Epstein et al. 1974; Yang et al. 1990). Hydrolysis products are listed in Table 36.

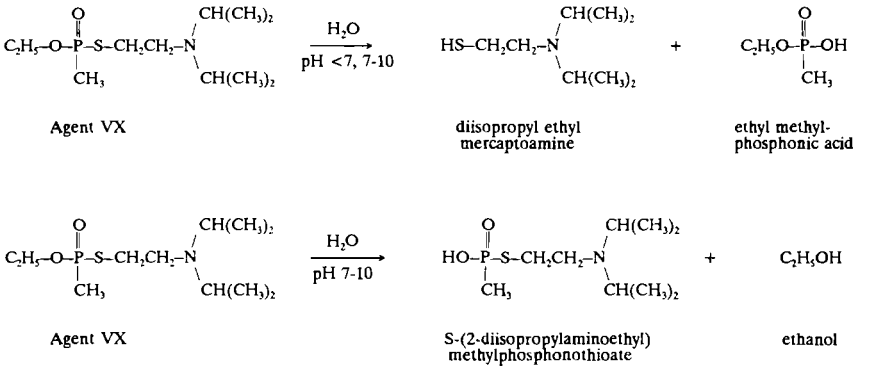


Fig. 3. Primary hydrolysis pathways of agent VX in the environment.

Unlike the G-agents, VX is relatively stable under acidic conditions. Although solubility is increased at lower temperatures, low temperatures decrease the rate of hydrolysis (Clark 1989). Trapp (1985) pointed out that, because of the low solubility of VX, hydrolysis data obtained under laboratory conditions may not be applicable to field conditions. VX in surface waters may sink and be adsorbed by sediment (Trapp 1985). A Henry's law constant of 8.19×10^{-9} atm-m^3/mole indicates that VX is essentially nonvolatile from water.

Although GA is readily soluble in water (98 g/L at 25 °C), the rate of hy-

Table 36. Agent VX hydrolysis products.

Product	Formula	CAS No.
Ethyl methylphosphonic acid	$C_3H_9PO_3$	1832-53-7
Diisopropyl ethyl mercaptoamine	$C_8H_{19}SN$	5842-07-9
S-(2-Diisopropylaminoethyl) methylphosphonothioate	$C_9H_{22}NSPO_2$	73207-98-4
Ethanol (ethyl alcohol)	C_2H_5OH	64-17-5
bis(2-Diisopropylaminoethyl) sulfide	$C_{16}H_{36}N_2S$	110501-56-9
bis(2-Diisopropylaminoethyl) disulfide	$C_{16}H_{36}N_2S_2$	65332-44-7
Ethyl methylphosphonothioic acid	$C_3H_9SPO_2$	18005-40-8
Diisopropylaminoethanol	$C_8H_{19}ON$	98-80-0
Methyl phosphonic acid[a]	CH_5PO_3	993-13-5

[a]Probable hydrolysis product; isolated from soil.

Sources: Clark (1989); DA (1974, 1988); Epstein et al. (1974); Kingery and Allen (1995); Small (1984); Yang et al. (1992).

drolysis is slow. At neutral pH and 25 °C, GA persists in water for 14–28 hr (Morrill et al. 1985); the half-life at 20 °C and pH 7.4 is approximately 8 hr (Clark 1989). The half-life in seawater at the same temperature is shorter, 4.5 hr (Clark 1989). Hydrolysis is temperature- and pH dependent, with the rate increasing with increasing temperature and under acidic and basic conditions (Clark 1989). Because of the formation of acidic products, a solution of GA will approach pH 4–5 as it hydrolyzes; the half-life of hydrolysis is maximum at pH 3–7.

Acidic and basic hydrolysis of GA result in different products (Fig. 4). Under acidic conditions, ethylphosphoryl cyanidate and dimethylamine are formed; under basic and neutral conditions, ethyl *N,N*-dimethylamido phosphoric acid and hydrogen cyanide are formed. Although the latter pathway is predominant, dimethylphosphoramidate, phosphorocyanidate, and dimethylphosphoramide cyanidate may also be formed (Sanches et al. 1993). The phosphorus-containing compounds are slowly hydrolyzed to phosphoric acid. Although theoretically possible, there is little likelihood of formation of a detectable amount of methyl phosphonic acid from GA. Hydrolysis products are listed in Table 37.

GB is unstable in the presence of water and is readily hydrolyzed by either acid or base to relatively nontoxic products. The hydrolysis rate of GB in water is temperature-, pH-, and water quality dependent (Clark 1989; Epstein 1974; Kingery and Allen 1995; Morrill et al. 1985). At 20 °C and the pH of natural waters where the half-life is a minimum, the half-life ranges from 461 hr (pH 6.5) to 46 hr (pH 7.5). At 25 °C, the half-life ranges from 237 hr (pH 6.5) to 24 hr (pH 7.5). The presence of acidic hydrolysis products increases the rate of

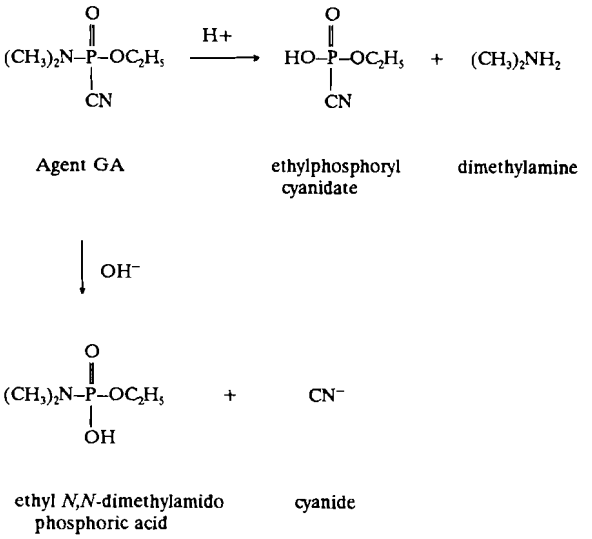


Fig. 4. Primary hydrolysis pathways of agent GA in the environment.

Table 37. Agents GA, GB, and GD: hydrolysis products.

Product	Formula	CAS No.
GA hydrolysis products		
Ethylphosphoryl cyanidate	$C_3H_6NPO_3$	117529-17-6
Dimethylamine	C_2H_7N	124-40-3
Ethyl *N,N*-dimethylamido phosphoric acid	$C_4H_{12}NPO_3$	2632-86-2
Hydrogen cyanide	HCN	74-90-8
Phosphorocyanidate	CH_2NPO_3	23852-43-9
Dimethylphosphoramidate	$C_2H_8NPO_3$	33876-51-6
Dimethylphosphoramide cyanidate	$C_3H_7N_2PO_2$	63917-41-9
GB hydrolysis products		
Isopropyl methylphosphonic acid	$C_4H_{11}PO_3$	1832-54-8
Hydrogen fluoride (hydrofluoric acid)	HF	7664-39-3
Methyl phosphonic acid	CH_5PO_3	993-13-5
Isopropyl alcohol	C_3H_8O	67-63-0
GD hydrolysis products		
Pinacolyl methylphosphonic acid	$C_7H_{17}O_3P$	616-52-4
Pinacolyl alcohol	$C_6H_{14}O$	464-07-3
Methyl phosphonic acid	CH_5PO_3	993-13-5

Sources: Clark (1989); DA (1974, 1988); MacNaughton and Brewer (1994); Sanches et al. (1993); Small (1984).

hydrolysis. A half-life of 8300 hr at 0 °C and pH of 6.5 indicates persistence at low temperatures. The rate of hydrolysis under natural conditions is accelerated by the presence of ions (dissolved solids) in solution. Metal cations such as copper and manganese in seawater also increase the rate of hydrolysis (Epstein 1974).

The fluorophosphonates GB and GD hydrolyze first through the loss of fluorine and second, more slowly, through the loss of the alkoxy group (Kingery and Allen 1995; MacNaughton and Brewer 1994). Under acidic conditions, the products of GB hydrolysis are isopropyl methylphosphonic acid and fluoride; the former slowly hydrolyzes to methyl phosphonic acid with the loss of isopropanol (Fig. 5). According to Clark (1989), alkaline hydrolysis results in isopro-

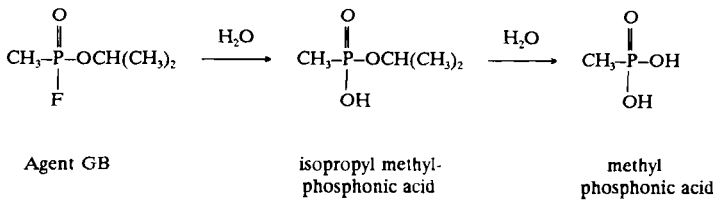


Fig. 5. Primary hydrolysis pathway of agent GB in the environment.

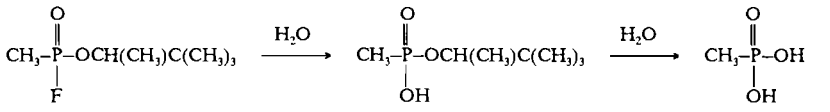
panol, methylfluorophosphonic acid and, with the loss of fluoride, methyl phosphonic acid. This pathway has not been confirmed in other studies. As noted, methyl phosphonic acid is stable in the environment (Sanches et al. 1993). Hydrolysis products are listed in Table 37.

GD dissolves in water but the rate of hydrolysis under neutral conditions is slow (Yang et al. 1992). Qualitatively, the hydrolysis of GD is similar to that of GA; however, the reaction rate is fivefold slower than that of GA, and GD has an estimated half-life of about 60 hr at pH 6 and 25 °C (Hambrook et al. 1971). The reaction is both acid- and base catalyzed, resulting in a hydrolysis curve similar to that of GA (Clark 1989). The primary hydrolysis product of GD is pinacolyl methylphosphonic acid, which slowly hydrolyzes, with the release of pinacolyl alcohol, to methyl phosphonic acid (Fig. 6) (Clark 1989; Kingery and Allen 1995). At pH >10, hydrolysis to pinacolyl methylphosphonic acid occurs within a few minutes (Yang et al. 1992). Because an acid is produced, the pH will decrease, lessening the rate of hydrolysis. GD stored at pH 6 for 8 wk had a pinacolyl methylphosphonic acid/methyl phosphonic acid ratio of 250 (Hambrook et al. 1971), which Kingery and Allen (1995) extrapolated to a half-life of 27 yr. The C–P bond is very resistant to hydrolysis. Hydrolysis products are listed in Table 37.

The calculated Henry's law constants of the three G-agents range from 1.52×10^{-7} atm-m^3/mole (GA) to 4.56×10^{-6} atm-m^3/mole (GD). These values indicate slow to essentially no volatilization from water.

Soil. Laboratory and field studies on the fate of nerve agents in soil indicate that disappearance results from a combination of processes including evaporation, hydrolysis, and microbial degradation. Soil types and properties and the presence and amount of soil moisture and bacteria greatly influence the rate of degradation.

Agent VX is less volatile (10.5 mg/m^3 at 25 °C) than the G-agents and does not evaporate readily. VX is moderately persistent on bare ground and may remain in significant concentrations for 2–6 d, depending on temperature, organic carbon content of the soil, and moisture (Sage and Howard 1989). In the laboratory, unstabilized VX of 95% purity decomposed at a rate of 5% a month at 22 °C (DA 1992b). Small (1984) used a surface deposition model to calculate



Agent GD — pinacolyl methylphosphonic acid — methyl phosphonic acid

Fig. 6. Primary hydrolysis pathway of agent GD in the environment.

a volatilization half-life of 13,000 hr for VX. Volatilization using a bulk soil model was five orders of magnitude slower.

Sanches et al. (1993) reported on a series of laboratory experiments performed in Canada in which soil samples were spiked with 60–100 μg VX/g of soil; analyses were performed during a period of 1 mon. The principal compounds detected during that time were diethyl methylphosphonate (an impurity and degradation product), bis(2-diisopropylaminoethyl) disulfide, and VX. Trace levels of other degradation products were present.

A series of reports on the hydrolysis of VX in soil (from Carroll Island, MD, a chemical agent test site) stored at room temperature were summarized by Small (1984). After 14 d of storage in closed containers, 2.5%–7.2% of initial levels of 10 mg/g of soil of VX were observed. Several different soils were used including a fine silty loam with pH of 6.5 and organic carbon content of 0.8%. Soil moisture did not greatly influence the disappearance. The disappearance of VX (1 mg/g of soil) from closed containers containing soil from Dugway Proving Ground was also studied; 21% was recovered after 3 d and 10% after 15 d.

In field studies conducted at Carroll Island, MD (reviewed by Small 1984), VX sprayed on soil decreased by about three orders of magnitude within 17–52 d. In an area of field tests at Dugway Proving Ground where soil levels before 1969 were as high as 6 mg/g, no VX was detected (detection limit, 0.4 μg/g) 10 yr later. The degradation product, methyl phosphonic acid, was detected at concentrations ranging from 14.9 to 23 μg/g; the acid was distributed uniformly through the 120-cm depth.

Approximately 3 wk after a report of the death of sheep at Skull Valley, near the Dugway Proving Ground, UT, on March 14, 1968, environmental samples from the grazing area were analyzed for VX (Sass et al. 1970). Analyses by a combination of analytical techniques revealed the presence of VX in snow and grass. Assuming 100% extraction efficiency, the snow sample contained 7–9 ng of intact VX per 400–500 g of water and the grass sample contained 4 μg of intact VX per 900 g of solid material. Agent concentrations present during the period of the sheep-kill incident could not be estimated.

VX was still present 2 and 4 wk after being sprayed on snow under normal Norwegian winter conditions. VX and other nerve agents did not penetrate deeply into the snow; snowfall covering the samples delayed evaporation (Johnsen and Blanch 1984; NMFA 1982, 1983).

Kaaijk and Frijlink (1977) and Verweij and Boter (1976) reported on the degradation (presumably by all processes) of VX in soil under laboratory conditions. Degradation was rapid with formation of ethyl methylphosphonic acid and diisopropyl ethyl mercaptoamine. After 1 d, the applied concentration of 0.2 mg/g soil decreased to 22% in humic sand and 2% in humic loam and clayey peat. Only 0.1% of applied VX was detectable in all soils after 3 wk. The half-life of ethyl methylphosphonic acid was 8 d; the degradation product was methyl phosphonic acid. Binding of the metabolites but not the parent compound correlated positively with the amount of organic matter in the soil. Small (1984)

pointed out that VX and GB degradation products sorb to soil depending on the soluble organic carbon content of the soil. Diethyl dimethylpyrophosphonate may also be formed from ethyl methylphosphonic acid in soil (Small 1984).

According to Morrill et al. (1985), evaporation is the primary mechanism for the loss of the nerve agents from soil. Although the G-agents are liquids under ordinary environmental conditions, their relatively high volatility at 25 °C (610 mg/m^3 for GA, 22,000 mg/m^3 for GB, and 3900 mg/m^3 for GD) and high vapor pressure permit them to be disseminated in vapor form. Because of its high volatility, GA is not expected to persist in soils. Results of a field trial with GA showed 10% evaporation in 0.27 hr and 90% evaporation in 4.66 hr (Morrill et al. 1985). Small (1984) used a surface deposition model to calculate a volatilization half-life of 7.7 hr for GB; based on similar structure, GD may be expected to act similarly. Volatilization rates calculated using a bulk soil model were three orders of magnitude slower.

Results of a field test with GB in Finland were reported by Sanches et al. (1993). Following application of 10 mg of GB over a 10×10 m area of moss (temperature 2.5 °–8 °C, humidity 60%–100%, wind speed 1–10 m/s), detectable concentrations (≥ 1 pg/dm^3) were found in the air for 9 d.

When GB was placed in closed containers of soil from Dugway Proving Ground at a concentration of 1 mg/g of soil, 13% was recovered in 3 d and 0.02% was recovered in 35 d (Small 1984). When GB was added to soils from Edgewood Arsenal and measured after 12 hr, 56% and 59% were observed on humus and loam of low moisture content, respectively; 42% and 40% were observed on humus and loam of higher moisture content, respectively. Low to nondetectable levels were present on the Edgewood Arsenal soils at 168 hr. The studies suggest that 90% of GB added to soil will be lost in the first 5 d.

Studies of the persistence of nerve agents performed at low temperatures (−1 °C) under actual field conditions in Norway show that they remain as liquids on snow (Johnsen and Blanch 1984; NMFA 1982, 1983). The agents were placed on the snow as "droplets." GA was present 2 wk after being sprayed on snow under normal Norwegian winter conditions, but was not measurable after 4 wk. GA and other nerve agents did not penetrate deeply into the snow; snowfall covering the samples delayed evaporation.

In the same study, droplets of GB deposited on the snow surface were removed by a combination of evaporation, which was dependent on wind speed, and hydrolysis. Within 5 hr, approximately 55% was removed by evaporation and 15% was removed by hydrolysis. Newly fallen snow protected droplets from evaporation. However, 2 and 4 wk after being sprayed on the snow, GB was still present. The hydrolysis product, isopropyl methylphosphonic acid, as well as the impurities diisopropyl methylphosphonate and dipinacolyl methylphosphonate were present, even after 4 wk.

In addition to hydrolysis, nerve agents may be transformed in soil by microbial degradation via O-dealkylation and C-dealkylation with resulting toxic products (Morrill et al. 1985). GA can also be biodegraded via nitrile hydrolysis, resulting in a nontoxic compound; N-dealkylation results in a compound that is

still toxic. D'Agostino and Provost (1992) analyzed soil contaminated by a leaking container of GA. In addition to GA, 16 related components including impurities and hydrolysis products were isolated: diethyl dimethylphosphoramidate, triethyl phosphate, ethyl tetramethyl phosphorodiamidate, tetramethylphosphorodiamidic cyanide, bis(ethyl dimethylphosphoramidic) anhydride, dimethyl phosphoric ethyl dimethylphosphoramidic anhydride, ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride, bis(ethyl dimethylamidophosphonyl) dimethylamidophosphonate, ethyl hydrogen dimethylphosphoramidate, diethyl hydrogen phosphate, ethyl dihydrogen phosphate, phthalate, and four unidentified components.

Although no direct information on biodegradation of the nerve agents in soil is available, enzymes capable of hydrolyzing organophosphorus esters, including some nerve agents, have been isolated from bacteria (Yang et al. 1992). However, the toxicity of these agents probably precludes direct biodegradation. It is known that all four nerve agents degrade to alkyl methylphosphonates by a variety of other mechanisms and then, slowly, to methyl phosphonic acid (with the possible exception of GA). The rate of disappearance of methyl phosphonic acid in environmental media is controlled by biodegradation (Kingery and Allen 1995). Soil types and properties greatly influence the rate of degradation.

Several strains of bacteria including *Pseudomonas testosteroni*, isolated from sewage samples, were capable of using methyl phosphonic acid as a phosphorus source but not as a carbon source (Small 1984). *Pseudomonas testosteroni* was also capable of metabolizing (via cleavage of the C–P bond) isopropyl methylphosphonic acid, a degradation product of GB, to an alkane and an inorganic phosphorus compound (Daughton et al. 1979).

2. *Ecotoxicology.* All the nerve agents are highly toxic to aquatic organisms, with 96-hr LC_{50} values (the normal duration of fish toxicity tests) of less than 1 mg/L. Weimer et al. (1970) measured the LT_{50} (time to lethality for 50% of the organisms) of VX for striped bass (*Morone saxatilis*) at 0.02 mg/L as 17.4 hr.

Death in goldfish (*Carassius auratus*) is usually preceded by a sudden change in behavior from quiescence to violent spasmodic action and is a more reliable indicator than cessation of movement. Hott and Alexander (1959) measured average times to spasm (ET_{50}) in 2-in. goldfish exposed to several concentrations of VX at a pH of 8.2 and a temperature of 25.5 °C. ET_{50} values at concentrations of 0.1, 0.3, and 1.0 mg/L were 15.0, 28.5, and 40.1 min, respectively. At 0.1 mg/L, time to death was estimated at 309 min.

Epstein (1956) reported the 20-min LC_{50} of GA in water for green sunfish (*Lepomis cyanellus*), fathead minnows (*Pimephales promelas*), and goldfish as 0.7, 0.6, and 1.3 mg/L, respectively. With minimum hydrolysis at a constant pH of 6, the 24-hr LC_{50} of GB for green sunfish was 0.002 mg/L and at a constant pH of 8, the LC_{50} was 0.0095 mg/L (Epstein 1956). Weiss and Botts (1957) measured the LT_{50} of GB ranging in concentrations from 0.01 to 50 µg/L to fish. At 24 °C, LT_{50} values ranged from 0.8 min at 50 mg/L to 6 hr at 0.01 mg/L for the fathead minnow, 0.95 min at 50 mg/L to 5.3 hr at 0.01 mg/L for the

green sunfish, and 1.5 min at 50 mg/L to 33 hr at 0.01 mg/L for the goldfish. "Toxic concentrations" for the three species were estimated to be 0.078 mg/L (goldfish), 0.06 mg/L (green sunfish), and 0.052 mg/L (fathead minnow). According to Epstein (1974), the data of Weiss and Botts (1957) give 20-min LC_{50} concentrations of 0.27, 0.3, and 0.5 mg/L for green sunfish, fathead minnow, and goldfish, respectively. For longer exposures, the hydrolysis rate of the agent becomes important.

Toxic effects (undefined) to vegetation occurred at solution culture concentrations of GB as low as 1 mg/L (Houle et al. 1972). Some terrestrial plants are sensitive to VX at concentrations as low as 10 ppm either in soil (Ross et al. 1983) or in aqueous solution (Worthley 1970). Although VX caused death of the plants, it did not prevent seed germination. Vegetation in agent test areas of Dugway Proving Ground in Utah and Aberdeen Proving Ground in Maryland, presumably exposed to both the agents and decomposition products (no measurements given), showed the same viability and growth as those in noncontaminated areas (McNamara and Leitnaker 1971).

Based on limited studies involving the relative toxicities of organophosphate pesticides in laboratory animals and wild birds, Sigal and Suter (1989) estimated median lethal doses (LD_{50}) of GB of 0.28 and 1.38 mg/kg for the mallard duck (*Anas platyrynchos*) and the ring-necked pheasant (*Phasianus colchicus*), respectively. Estimated LD_{50} values for VX were 0.012 and 0.18 mg/kg for the two species, respectively. Chemicals with LD_{50} values less than 1 mg/kg in laboratory animals are considered extremely toxic. According to Sigal and Suter (1989), limited evidence indicates no accumulation of nerve agents in food chains beyond herbivores.

C. Agent L (Lewisite)

1. Environmental Fate. When manufactured by normal processes, commercial lewisite is composed of cis and trans isomers in the ratio of 10 : 90 and several impurities including bis(2- chlorovinyl)chloroarsine and tris(2-chlorovinyl)arsine (Rosenblatt et al. 1975). The chemical and physical properties of the two isomers are similar.

Air. Lewisite is more volatile than sulfur mustard agents and is used as a moderate irritant vapor over greater distances than sulfur mustard (Watson and Griffin 1992). Although no data on its fate in the atmosphere were located, its UV absorption spectrum at 200–350 nm is greater than that of other nonstockpile chemical agents (Rewick et al. 1986), indicating that some photodegradation may take place. Hydrolysis may also occur in the gas phase (MacNaughton and Brewer 1994).

Water. Although lewisite is only slightly soluble in water, 0.5 g/L (Rosenblatt et al. 1975), hydrolysis resulting in the formation of lewisite oxide and HCl is rapid. The hydrolysis is complex, with a number of reversible reactions (Clark

1989; Epstein 1956; MacNaughton and Brewer 1994; Rosenblatt et al. 1975). In slightly acidic solutions, lewisite first undergoes a rapid and reversible reaction to the dihydroxy arsine:

$$Cl{-}CH{=}CH{-}AsCl_2 + 2H_2O \rightleftarrows Cl{-}CH{=}CH{-}As(OH)_2 + 2\ HCl$$

Reaction of the dihydroxy compound is slower, eventually forming lewisite oxide (chlorovinyl arsenous oxide) and polymerized lewisite oxide:

$$Cl{-}CH{=}CH{-}As(OH)_2 \rightleftarrows H_2O + Cl{-}CH{=}CH{-}AsO + (Cl{-}CH{=}CH{-}AsO)_n$$

The reaction is driven to the right because lewisite oxide and polymerized lewisite oxide are insoluble. In basic solution, the trans-lewisite isomer is cleaved by the hydroxyl ion to give acetylene and sodium arsenite; this occurs even at low temperatures (Clark 1989; Rosenblatt et al. 1975). cis-Lewisite must be heated above 40 °C to react with NaOH to yield vinyl chloride, sodium arsenite, and acetylene (Rosenblatt et al. 1975). In aqueous solution, the cis-isomer undergoes a photoconversion to the trans-isomer (Rosenblatt et al. 1975). On standing in water and in the presence of oxidizers naturally present in the environment, the toxic trivalent arsenic of lewisite oxide is oxidized to the less toxic pentavalent arsenic (Epstein 1956). Regardless of the degradation pathway, arsenical compounds persist in the environment.

A Henry's law constant of 3.2×10^{-4} atm-m^3/mole indicates the potential for significant volatilization from water. However, the rapid rate of hydrolysis may reduce the significance of this fate pathway.

Soil. Lewisite applied to soil may rapidly volatilize or be converted to lewisite oxide through exposure to soil moisture (Rosenblatt et al. 1975). However, its low water solubility indicates intermediate persistence in moist soil (Watson and Griffin 1992). Both lewisite and lewisite oxide may be slowly oxidized to 2-chlorovinylarsonic acid ($C_2H_4AsClO_3$) (Rosenblatt et al. 1975). Suggested pathways of microbial degradation in soil include epoxidation of the C=C bond and reductive dehalogenation and dehydrohalogenation (Morrill et al. 1985). The latter pathways result in toxic metabolites because of the epoxy bond and arsine group. In addition, residual hydrolysis would result in arsenical compounds. Although lewisite probably does not bioaccumulate through food chains, arsenic may (Rosenblatt et al. 1975).

2. *Ecotoxicology.* Data are limited to a few historical reports. In 30-d tests, the threshold for lethality for several aquatic organisms were 0.2 mg/L (small black bullheads), 0.5 mg/L (bluegill sunfish), <2.0 mg/L (largemouth bass), and 0.5 mg/L (tadpoles) (Buswell et al. 1944; Price and von Limbach 1945). In another study, sunfish exposed to 6.5 mg/L for 24 hr showed signs of stress but no deaths (Bauer et al. 1955). Buswell et al. (1944) compared the toxicity of lewisite to that of arsenite ($NaAsO_2$) and found that the 30-d lethality threshold of arsenite for black bullheads was much greater, 25 mg/L.

The toxicity of lewisite to a variety of phytoplankton and aquatic plants (water milfoil, parrot's feather, and water crowfoot) was tested in static 30-d tests at concentrations of 5 and 50 mg/L (Buswell et al. 1944). At 5 mg/L, lewisite inhibited the growth of the phytoplankton, and the water milfoil and water crowfoot died; at 50 mg/L, all plants died. Lewisite vapor is extremely phytotoxic and has been implicated in the death of vegetation in lewisite shell target areas (Armstrong et al. 1928).

D. Agent CK (Cyanogen Chloride)

1. Environmental Fate

Air. At ambient temperature (20 °C) cyanogen chloride is an extremely volatile liquid. No data on its fate in the atmosphere were located.

Water. Because of its extreme volatility and relatively rapid hydrolysis in water, cyanogen chloride is not expected to persist in surface waters. Hydrolysis half-lives range from 1 min at 45 °C to 10 hr at 5 °C (Bailey and Bishop 1970). Kononen (1988) calculated a half-life of 5.25 hr at 20 °C and pH 8.64. According to APHA (1975), cyanogen chloride may persist for 24 hr at a pH of 9.0 if no excess chlorine is present. Cyanogen chloride undergoes considerable hydrolysis at alkaline pH to form cyanic (HOCN) and hydrochloric acids; the same products are formed at a slower rate at acidic and neutral pH values (Clark 1989):

$$ClCN + OH^- \rightarrow HOCN + Cl^-$$

$$ClCN + H^+ \rightarrow HOCN + H^+ + Cl^-$$

$$ClCN + H_2O \rightarrow HOCN + H^+ + Cl^-$$

Cyanogen chloride from sources other than chemical agents may be present in natural waters. Cyanogen chloride may be formed in natural waters by the action of active chlorine on dissolved or suspended organic matter in the presence of ammonia or amines. Humic acid in solution at a concentration of 1 mg/L and 2–5 mg/L ammonia reacted with 18 mg/L of dissolved chlorine to yield 8 µg/L cyanogen chloride; 42 mg/L of dissolved chlorine formed 20 µg/L of cyanogen chloride (Ohya and Kanno 1985). Cyanogen chloride is also formed by the action of active chlorine on cyanide ion or hydrocyanic acid in dilute aqueous solution (Clark 1989). Disinfection of drinking water by chlorination results in the formation of chlorinated byproducts including cyanogen chloride. Concentrations of cyanogen chloride in finished drinking water from 11 water utilities ranged from less than 0.3 (the method detection limit) to 13.7 µg/L (Flesch and Fair 1989). Cyanogen chloride was preferentially produced in chloraminated water; of 435 utilities that provide water with a free chlorine residual, the median cyanogen chloride value was 0.4 µg/L, whereas in those that deliver chloraminated water, the median value was 1.6 µg/L (Krasner et al. 1989a,b).

Soil. No data on the fate of cyanogen chloride in soil were located; however, its fate in soil would probably be similar to that in water, i.e., volatilization and hydrolysis.

2. *Ecotoxicology.* Cyanogen chloride is extremely toxic to aquatic organisms. The 48-hr LC_{50} for *Daphnia magna* neonates (<24 hr old) in a static test was 29 µg/L (as CN^-); that for individuals ≤5 d old was 65 µg/L (Kononen 1988). These values may be overestimates because measurable concentrations of cyanogen chloride were not present at the end of the experiment. These values are considerably less than those of free cyanide as the 48-hr LC_{50} for free cyanide for this same species was 3.4 mg/L (Meinck et al. 1956). Because of the instability of cyanogen chloride in water, Sabourin et al. (1987) tested *Daphnia magna* in a flow-through system using measured concentrations. In this test system, the 48-hr LC_{50} of young daphnids (<24 hr old) was 22.5 µg/L.

Verschueren (1996) reported a 6- to 8-hr lethal concentration of cyanogen chloride for goldfish of 1 mg/L. In a flow-through system and using measured concentrations, the 96-hr LC_{50} of fathead minnows was 147.3 µg/L (Sabourin et al. 1987). This result is close to the 96-hr LC_{50} values of free cyanide for fathead minnows of 120 and 110 µg/L at pH values of 8.29 and 8.67, respectively, as reported by Broderius et al. (1977).

XVI. Discussion and Conclusions

The oral Reference Doses (RfD_o) calculated in this report for chemical warfare agents, together with the Uncertainty and Modifying Factors used in their derivation, are listed in Table 38. Data were insufficient for deriving RfD_os for agents HT, T, and HN2.

The toxicity data bases for the chemical warfare agents evaluated in this report, particularly in terms of subchronic or chronic studies, vary considerably both in completeness and in applicability to RfD derivation. Special protocols were used in toxicity testing of these agents because of their acute toxicity and, in some cases, because of their physicochemical characteristics and reactivity. Consequently, the resulting data were often less than ideal for deriving RfD_os. For example, the only available long-term toxicity study for agent GA is an intraperitoneal injection study, and one of only two relevant subchronic studies on agent VX utilized subcutaneous injections. In the case of agent GB, the only available subchronic oral studies are tests in which the agent was mixed with a stabilizer. For both agents GB and GD, as well as sulfur mustard and lewisite, testing protocols involved oral gavage dosing. Gavage dosing with vesicants introduces obvious complications in assessing potential human health risks associated with more likely exposure pathways. Similarly, gavage dosing with the nerve agents is likely to result in different toxicokinetics compared to that resulting from less concentrated exposures through food or drinking water. Combining the test compound with another chemical, as in the case of GB with stabilizer, introduces several possibilities: altered toxic responses, different critical

Table 38. Summary of estimated RfDs and total Uncertainty Factors (UFs) for chemical warfare agents.

Chemical agent	RfD_e ($\mu g\ kg^{-1}d^{-1}$)	Total UF	Reference for critical study
HD	7×10^{-3}	3000	Sasser et al. (1989b)
HT	NV	—	—
T	NV	—	—
HN2	NV	—	—
VX	6×10^{-4}	100	Rice et al. (1971)
GA	4×10^{-2}	3000	Bucci et al. (1992a)
GB	2×10^{-2}	3000	Bucci and Parker (1992)
GD	4×10^{-3}	3000	Bucci et al. (1992c)
Lewisite	NV[a]	—	—
Cyanogen chloride[b]	30	30	Ministry of Health, Mozambique (1984a,b)
Cyanogen chloride[c]	30	300	NTP (1993)

NV, not verifiable; insufficient data for calculating an RfD.

[a]It is recommended that the RfD_e for inorganic arsenic, 0.3 $\mu g\ kg^{-1}d^{-1}$, be used as a surrogate for lewisite.

[b]Derived from human epidemiological data.

[c]Derived from animal data.

effects, or NOAELS and LOAELS different from those which might have resulted if the pure compound had been tested. Deriving RfDs from nonoral data, as in the case of GA (i.p. data), introduces difficulties in extrapolating across different exposure routes. Such deficiencies in the original data were taken into account in deriving the RfD_es.

Because of the critical need for deriving some measure of the subchronic/chronic toxic potency of these compounds, RfD_es were calculated even in those cases in which we were aware of deficiencies in the available data. Our best scientific judgment (and that of the individuals and groups reviewing these values) was used in interpreting the toxicity data and applying the appropriate Uncertainty Factors and Modifying Factors. In several instances this procedure involved nonstandard approaches, such as using acute toxicity ratios to estimate an oral NOAEL from an intraperitoneal NOAEL (agent GA), or using statistical analysis of dose–response data to modify the NOAEL-to-LOAEL Uncertainty Factor (agent VX). In analyzing all the available data, our primary goal was to reduce the uncertainties associated with the RfD_es, particularly with regard to the use of the standard default values of 10. A major issue in this regard is our evaluation of the test data for agent VX and our conclusion that humans would not be any more sensitive to the agent than the test species, sheep. We are aware that this conclusion contradicts the standard assumption that humans should be

considered 10 times more sensitive than a laboratory species, but we believe that the test data support our position.

It is of interest to point out that in all those cases for which we had data, the critical effect (i.e., the effect identified by the LOAEL) was the same as that associated with the acute toxicity of the chemicals, namely, cholinesterase inhibition for the nerve agents and vesicant activity for HD and lewisite. This concurrence is not unusual for such acutely toxic compounds. Therefore, it would be expected that within each group, the RfD_es would reflect the relative potency of these compounds to produce acute effects. For example, for the nerve agents, VX is much more acutely toxic than the G-agents, and within the G-agents, GD is more acutely toxic than GA or GB. The proposed RfD_es for these compounds reflect the same relative toxicity. The nerve agent RfD_es are also consistent with RfD_es for other anticholinesterase organophosphate compounds, the RfD_e for agent VX being lower than that for any other organophosphate RfD documented on EPA's Integrated Risk Information System. The "reasonableness" of the RfD_es was also evaluated by comparison with human data for the same compounds. Where the human data were adequate for comparison, these indicated that the RfD_es provide a wide margin of safety.

It should be noted that the proposed RfD_es have undergone extensive review by individuals and working groups knowledgeable about the toxicity of the compounds and the process of deriving RfD_es. With the exception of one compound, lewisite, the proposed RfD_es have been reviewed and approved by the Material/Chemical Risk Assessment (MCRA) Working Group of the Environmental Risk Assessment Program (ERAP). In the case of lewisite, MCRA concluded that the overall data base was not adequate for deriving an RfD_e, and the recommendation was made that the RfD for inorganic arsenic be used as a surrogate for lewisite. It should be emphasized, however, that even with the identified deficiencies, the RfD_e calculated for lewisite in this report is not substantially different from that of inorganic arsenic. Therefore, the use of the arsenic RfD does not in any way compromise risk assessments conducted on this compound.

Application of the proposed RfD_es in health risk assessments requires a realistic analysis of the environmental fate of these chemicals. For some of the agents the rate of degradation in the environment is sufficiently rapid that the chemicals are unlikely to pose a chronic hazard unless they are continuously released from a point source. Cyanogen chloride is so highly volatile that its presence in soil or drinking water is highly unlikely. The G-agents are volatile, soluble in water, and also subject to hydrolysis and biodegradation such that their expected environmental half-lives are measured in days to weeks. None of these compounds is likely to be present for a sufficiently long period of time to represent a chronic health hazard.

The agents that can be considered environmentally persistent, in varying degrees, are VX, sulfur mustard, and lewisite. Agent VX exhibits very low volatility and a slow rate of hydrolysis; its half-life in soil may be measured in weeks to months, depending on environmental conditions. Sulfur mustard may persist

in soils for several years when not exposed to weathering conditions. Although lewisite degrades rapidly, its degradation products, such as the equally toxic lewisite oxide, are more persistent, and its ultimate degradation product, inorganic arsenic, will remain in the environment.

Although these three agents may remain in soils for varying lengths of time, the threat of groundwater contamination does not appear to be significant. In the case of agent VX, hydrolysis and biodegradation processes, although slow, are likely to limit the amount of VX reaching subsurface aquifers, particularly in view of the relatively long time required for percolation through the unsaturated zone. Furthermore, although slow, the hydrolysis of VX in water (half-life, 400–1000 hr) is relatively rapid compared to the slow lateral flow rates of groundwater (30–300 m/yr). Transport of sulfur mustard through soils is expected to be limited by the formation of thiodiglycol-mustard aggregates, which would prevent further dissolution of the agent into soil pore water, and any sulfur mustard that does become dissolved would be subject to rapid hydrolysis. The insolubility of lewisite oxide would limit transport of this degradation product into groundwater, and movement of arsenic through soils to groundwater is not expected to occur unless the arsenic is present in the form of a soluble salt.

Finally, it should be noted that the proposed RfD_cs, like those for any other industrial chemical, are designed to be protective for chronic exposures lasting for 7 years to a lifetime. An added margin of safety is afforded in those situations where the duration of exposure is expected to be less than 7 years.

Summary

Health risk assessments for sites contaminated with chemical warfare agents require a comparison of the potential levels of exposure with a characterization of the toxic potency of each chemical. For noncancer health effects, toxic potency is expressed in terms of Reference Doses (RfD).

A RfD is a daily exposure level or dose (usually expressed in units of milligrams of chemical per kilogram body weight per day) for the human population, including sensitive subpopulations, that is likely to be without an appreciable risk of deleterious effects. A daily exposure at or below the RfD is not likely to be associated with health risks, but as the amount of chemical that an individual is exposed to increases above the RfD, the probability that an adverse effect will occur also increases.

A RfD is derived by first examining the available human or animal toxicity data to identify a dose or exposure that corresponds to a no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL). The NOAEL is the exposure level at which there are no statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects may be produced at this level, but they are not considered to be adverse if they do not result in functional impairment or pathological lesions that affect the performance of the whole organism or which reduce an organism's ability to cope with additional chal-

lenge. The LOAEL is the lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. If only a LOAEL is identified by the toxicity data, a NOAEL is estimated by dividing the LOAEL by a factor no greater than 10. This extrapolation factor of 10 or less is termed the LOAEL-to-NOAEL Uncertainty Factor (UF_L).

The NOAEL is also adjusted by the application of other Uncertainty Factors, including (1) a $UF_H \leq 10$ to ensure that the resulting RfD protects segments of the human population that may be more sensitive to the chemical than the average person; (2) a $UF_A \leq 10$ to extrapolate from the experimental animal species to humans; (3) a $UF_S \leq 10$ to extrapolate from an experimental subchronic exposure study to a potential chronic exposure; and (4) a $UF_D \leq 10$ to ensure that the resulting RfD is protective for all possible adverse effects, particularly those that may not have been adequately evaluated in the available studies. A Modifying Factor (MF), based on a qualitative professional assessment of the data, may also be used to account for other factors (e.g., deficiencies in the critical study) that are not adequately covered by the standard Uncertainty Factors.

1. Agent HD (Sulfur Mustard). $RfD_c = 7 \times 10^{-6}$ mg kg^{-1} d^{-1}. A LOAEL was identified in a two-generation reproductive toxicity study conducted in rats. A total uncertainty factor of 3000 was applied to account for protection of sensitive subpopulations (10), animal-to-human extrapolation (10), LOAEL-to-NOAEL extrapolation (3), and extrapolation from a subchronic to chronic exposure (10). A LOAEL-to-NOAEL UF of 3, instead of the default value of 10, was used because the critical effect (stomach lesions) was considered to be "mild" in severity and may have been enhanced by the vehicle used (sesame oil in which sulfur mustard is fully soluble) and the route of administration (gavage), which is more likely to result in localized irritant effects. The key study did identify a toxic effect that is consistent with the vesicant properties of sulfur mustard. In none of the other available studies was there any indication of a different effect occurring at a lower exposure level.

2. Agent VX. $RfD_c = 6 \times 10^{-7}$ mg kg^{-1} d^{-1}. A LOAEL was identified in a 56-d study in sheep. A total uncertainty factor of 100 was applied to account for protection of sensitive subpopulations (10), LOAEL-to-NOAEL extrapolation (3), and extrapolation from a subchronic to chronic exposure (3). An uncertainty factor was not used to extrapolate from the animals to humans because there was evidence that humans are not any more sensitive to VX than sheep. An uncertainty factor of 3 rather than the default value of 10 was used to extrapolate from a subchronic to chronic exposure because the experimental data indicated that the effect stabilized by the end of the study (i.e., the effect did not continue to accumulate with increasing exposure time). A LOAEL-to-NOAEL uncertainty factor of 3 was used because the endpoint, cholinesterase inhibition, was not associated with any physical signs of clinical toxicity, and because the

NOAEL based on the default UF would have been substantially lower than that predicted from an analysis of the dose–response data. The overall data base for VX was not considered to have any major deficiencies that would warrant the use of a UF_D greater than 1.

3. Agent GA (Tabun). $RfD_e = 4 \times 10^{-5}$ mg kg^{-1} d^{-1}. A NOAEL was identified in a 90-d study in rats. A total uncertainty factor of 3000 was applied to account for protection of sensitive subpopulations (10), animal-to-human extrapolation (10), extrapolation from a subchronic to chronic exposure (3), and incomplete data base (3). An uncertainty factor of 3 was used to extrapolate from a subchronic to chronic exposure because of the unlikelihood that the LOAEL would have been substantially lower if the exposure had been chronic. A LOAEL-to-NOAEL uncertainty factor was not needed because a NOAEL was used in the derivation. The data base for GA lacks a multigeneration reproductive toxicity study, but because the available evidence indicates that organophosphate cholinesterase inhibitors such as GA are not likely to be reproductive toxins, the missing study was not considered critical. Therefore, a UF_D of 3, not the default value of 10, was applied. A Modifying Factor of 3 was applied because the key study involved a nonoral exposure route (intraperitoneal injections).

4. Agent GB (Sarin). $RfD_e = 2 \times 10^{-5}$ mg kg^{-1} d^{-1}. A LOAEL was identified in a 90-d oral study in rats. A total uncertainty factor of 3000 was applied to account for protection of sensitive subpopulations (10), animal-to-human extrapolation (10), LOAEL-to-NOAEL extrapolation (3), extrapolation from a subchronic to chronic exposure (3), and incomplete data base (3). An uncertainty factor of 3 rather than 10 was used to extrapolate from a subchronic to chronic exposure because of the unlikelihood that the LOAEL would have been substantially lower if the exposure had continued for a longer period of time. The LOAEL-to-NOAEL uncertainty factor of 3 was used because the endpoint, cholinesterase inhibition, was not associated with any physical signs of clinical toxicity. A pilot multigeneration reproductive toxicity study on GB was inconclusive; however, because the available evidence indicates that organophosphate cholinesterase inhibitors such as GB are not likely to be reproductive toxins, the lack of definitive results was not considered critical. Therefore, a UF_D of 3, not the default value of 10, was applied.

5. Agent GD (Soman). $RfD_e = 4 \times 10^{-6}$ mg kg^{-1} d^{-1}. A LOAEL was identified in a 90-d oral study in rats. A total uncertainty factor of 3000 was applied to account for protection of sensitive subpopulations (10), animal-to-human extrapolation (10), LOAEL-to-NOAEL extrapolation (3), extrapolation from a subchronic to chronic exposure (3), and incomplete data base (3). An uncertainty factor of 3 rather than the default value of 10 was used to extrapolate from a subchronic to chronic exposure because of the unlikelihood that the LOAEL would have been substantially lower if the exposure had continued for a longer period of time. A LOAEL-to-NOAEL uncertainty factor of 3 was used because

the endpoint, cholinesterase inhibition, was not associated with any physical signs of clinical toxicity. The data base for GD lacks reproductive and developmental toxicity studies, but because the available evidence indicates that organophosphate cholinesterase inhibitors such as GD are not likely to be reproductive or developmental toxins, the missing studies were not considered critical. Therefore, a UF_D of 3, not the default value of 10, was applied.

6. Lewisite. $RfD_e = 1 \times 10^{-4}$ mg kg^{-1} d^{-1}. A NOAEL was identified in a multigeneration study conducted on rats. A total uncertainty factor of 3000 was applied to account for protection of sensitive subpopulations (10), animal-to-human extrapolation (10), extrapolation from a subchronic to chronic exposure (10), and data base deficiencies (3).

The proposed RfD_e for lewisite underwent preliminary review (July 10–12, 1996) by the Material/Chemical Risk Assessment (MCRA) Working Group of the Environmental Risk Assessment Program (ERAP). The MCRA Working Group of ERAP represents multiagency (EPA, DOD, and DOE) input by individuals experienced in deriving and validating toxicity values. The MCRA Working Group agreed that the critical toxic effect observed in the lewisite studies (forestomach lesions) appears to be an artifact of administration and that the overall database for lewisite is not robust. Although it was recognized that the structure of lewisite might imply toxic activity differing from inorganic arsenic, it was the consensus of the MCRA Working Group that the lewisite RfD_e be considered not verifiable because of data deficiencies, and that the existing RfD for inorganic arsenic (3×10^{-4} mg kg^{-1} d^{-1}) be used as a surrogate. This approach is considered valid and justifiable inasmuch as the inorganic arsenic RfD and the proposed lewisite RfD_e are similar (0.0003 mg kg^{-1} d^{-1} for inorganic arsenic and 0.0001 mg kg^{-1} d^{-1} for lewisite), and the fact that lewisite in environmental media is degraded to inorganic arsenic.

7. Cyanogen Chloride (CK). $RfD_e = 3 \times 10^{-2}$ mg kg^{-1} d^{-1}. Data were not available to derive an RfD_e directly from studies conducted on CK. Because the systemic toxicity of CK results from its transformation to free cyanide, CK is expected to elicit the same toxic effects as cyanide. Therefore, an oral RfD_e for CK was derived from studies conducted on cyanide. Two studies were considered cocritical in the derivation of the RfD_e: one animal study and one human epidemiological study. In the animal study, a NOAEL was identified and a total uncertainty factor of 300 was applied to account for protection of sensitive subpopulations (10), animal-to-human extrapolation (10), and extrapolation from a subchronic to chronic exposure (3). A full factor of 10 was not used for the latter UF because chronic oral data were also available which showed that the subchronic NOAEL was adequately protective. In the human epidemiological study, a LOAEL was identified and a total uncertainty factor of 30 was applied to account for extrapolation from a subchronic to chronic exposure (3) and extrapolating from a LOAEL to a NOAEL (10). A uncertainty factor for sensitive human subpopulations was not used because the subject population

consisted of starving women and children, a group likely to be highly susceptible to cyanide toxicity. Although the affected population was chronically exposed to cassava in their diet (the source of the cyanide); a subchronic to chronic UF of 3 was used because the maximum cyanide intake occurred during a relatively short drought period. The overall data base for cyanide was considered to be adequate for deriving an RfD_c for cyanogen chloride.

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Appendix A: List of Acronyms and Abbreviations

ACGIH	American Conference of Governmental and Industrial Hygienists
ACh	acetylcholine
AChE	acetylcholinesterase
Agent AC	hydrogen cyanide; CAS # 0074–90-8
Agent BZ	3-quinuclidinyl benzilate; CAS # 6581–06-2
Agent CS	*O*-chlorobenzylidene malononitrile; CAS # 2698–41-1
Agent CG	phosgene (carbonyl chloride); CAS # 0075–44-5
Agent CK	cyanogen chloride; CAS # 0506–77-4
Agent CN	2-chloroacetophenone; CAS # 532–27-4
Agent CX	phosgene oxime (dichloroformoxime)
Agent GA	tabun (ethyl dimethylamidocyanophosphate); CAS # 77–81-6
Agent GB	sarin (isopropyl methylphosphonofluoridate); CAS # 107–44-8
Agent GB type I	sarin with the stabilizer tributylamine
Agent GB type II	sarin with the stabilizer diisopropylcarbodimide
Agent GD	soman (pinacolyl methylphosphonofluoridate); CAS # 96–64-0
Agent H	sulfur mustard, production run; 70% HD and 30% sulfur impurities
Agent HD	sulfur mustard, distilled [bis(2-chlorethyl)sulfide]; CAS # 505–60-2
Agent HN1	nitrogen mustard [bis(2-chloroethyl)ethylamine]; CAS # 538–07-8
Agent HN2	nitrogen mustard [bis(2-chloroethyl)methylamine]; CAS # 51–75-2

Agent HN3	nitrogen mustard [tris(2-chloroethyl) amine]; CAS # 555–77-1
Agent HT	sulfur mustard mixture (approximately 60% agent HD and <40% agent T, plus sulfur impurities)
Agent L	lewisite [dichloro(2-chlorovinyl)arsine]; CAS # 541–25-3
Agent PS	chloropicrin; CAS # 0076–06-2
Agent T	bis[2-(2-chloroethylthio)-ethyl] ether; CAS # 63918–89-8
Agent VX	*O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothioate; CAS # 50782–69-9
Agent WP	white phosporus; CAS # 7723–14-0
ANL	acute nonlymphocytic leukemia
ANOVA	analysis of variance
APHA	American Public Health Association
AR	Army Regulation
ATSDR	Agency for Toxic Subtances and Disease Registry
CAS	Chemical Abstracts Service
CDC	Centers for Disease Control
CD rat	cesarian-derived Sprague-Dawley rat
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act of 1980, PL 96–510
cf	confidence limits
CFR	Code of Federal Regulations
CF rat	Carworth Farms rat
ChE	cholinesterase
ChE_{50}	dose producing 50% inhibition of cholinesterase
CNS	central nervous system
COT	Committee on Toxicology
CSEPP	Chemical Stockpile Emergency Preparedness Program
CSM	chemical surety materials
CW	chemical weapons
CWC	Chemical Weapons Convention
DA	U.S. Department of the Army
DA	Pam Department of the Army Pamphlet
DERP	Defense Environmental Restoration Program
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	U.S. Department of Defense
DOE	U.S. Department of Energy
DTIC	Defense Technical Information Center
EC_{50}	effective concentration producing a response in 50% of the test animals
ED_{50}	effective dose producing a response in 50% of the test animals

ET_{50}	effective time to a response in 50% of the test animals
EEG	electroencephalogram
EKG	electrocardiogram
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
ERAP	Environmental Risk Assessment Program
FR	Federal Register
FUDS	Formerly Used Defense Sites
GSH	glutathione
HA	Health Advisory
HEAST	Health Effects Assessment Summary Table
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
HMS	hexose monophosphate shunt
HSDB	Hazardous Substances Data Base
I_{50}	concentration causing 50% enzyme inhibition
IARC	International Agency for Research on Cancer
ICR	Institute for Cancer Research
ICt_{50}	concentration × exposure time incapacitating to 50% of exposed individuals
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IRP	Installation Restoration Program
IU	International units
i.v.	intravenous
k_i	bimolecular rate constant for reaction of organophosphates with acetylcholine
LC_{50}	concentration lethal to 50% of test animals; median lethal concentration in units of mg/m^3
LC_{Lo}	lowest lethal concentration
LCt_{50}	concentration × exposure period lethal to 50% of test animals
LD_{50}	dose lethal to 50% of test animals
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
LEL	lowest-effect level
LT_{50}	time to lethality for 50% of test animals
MCRA	Material/Chemical Risk Assessment Working Group
MF	Modifying Factor
$mg\text{-}min/m^3$	milligram × minutes per cubic meter
mM	millimoles
mol wt	molecular weight
NAD^+	nicotinamide adenine dinucleotide
NCTR	National Center for Toxicological Research

NLM	National Library of Medicine
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NSCM	nonstockpile chemical materiel
NTE	neuropathy target esterase (formerly called neurotoxic esterase)
NTIS	National Technical Information Service
NTP	National Toxicology Program
OPIDN	organophosphate-induced delayed neuropathy
OSHA	Occupational Safety and Health Administration
PADPRP	poly(ADP-ribose) polymerase
PL	Public Law
p.o.	per os (by mouth)
QL	*O*-ethyl-*O'*-(2-diisopropylaminoethyl)-methylphosphonite
RBC	red blood cell
RBC-ChE	red blood cell cholinesterase
REM	rapid eye movement
RfD	Reference Dose
RfD_e	estimated Reference Dose (derived in this report)
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act of 1986, PL 99–499
SCE	sister chromatid exchange
SD	standard deviation
SEM	standard error of the mean
SERDP	Strategic Environmental Research Development Program
SF	slope factor
SFEMG	single fiber electromyography
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SMR	standardized mortality ratio
S-9	mixed function oxidase preparation
TOXLINE	National Library of Medicine data retrieval system
TOXLIT	National Library of Medicine data retrieval system
TSCA	Toxic Substances Control Act of 1976, as amended, PL 94–469
TWA	time-weighted average
UF	Uncertainty Factor
UF_A	Uncertainty Factor up to 10 to be used when extrapolating from animal data to humans and based on the assumption that humans are likely to be more sensitive than animals
UF_D	Uncertainty Factor up to 10 to be used when the avail-

	able data do not adequately address all possible adverse outcomes in humans
UF_H	Uncertainty Factor up to 10 to account for variation in the general population, and intended to protect sensitive subpopulations
UF_L	Uncertainty Factor up to 10 to be used when a suitable NOAEL is not available and when a LOAEL is used instead
UF_S	Uncertainty Factor up to 10 to be used when extrapolating from a subchronic study to derive a chronic RfD_c
USACHPPM	U.S. Army Center for Health Promotion and Preventive Medicine
USACMDA	U.S. Army Chemical Materiel Destruction Agency
USAEC	U.S. Army Environmental Center
USATHAMA	U.S. Army Toxic and Hazardous Materials Agency
vol%	volume percent
WHO	World Health Organization
wt%	weight percent
μM	micromoles

Appendix B: Glossary of Technical Terms

Absorbed dose—amount of a chemical that enters the systemic circulation of an exposed organism

Absorption factor—fraction of a chemical making contact with an organism that enters the blood

Acanthosis—benign overgrowth of the prickle-cell layer of the skin

Acetylcholine—chemical transmitter of nerve impulses across synapses

Acidosis—pathological condition resulting from accumulation of acid in, or loss of base from, the blood or body tissues

Acute exposure—one-time or short-term exposure (usually within a 24-hr period) that may or may not cause a health problem

Anaphylaxis—severe allergic reaction

Anemia—reduction in the oxygen-carrying capacity of the blood

Anorexia—loss of appetite

Anoxia—decrease in oxygen levels in body tissues below physiological levels

Ataxia—failure of muscular coordination; irregularity of muscular action

Atrial—referring to an upper chamber of the heart

Blepharospasm—tonic spasm of the eye muscles causing closure of the eyelids

Bowen's disease—intraepidermal squamous cell carcinoma

Bradycardia—slowness of the heartbeat as evidenced by slowing of pulse rate to less than 60

Bronchiectasis—chronic dilatation of the bronchi

Bronchopneumonia—inflammation of the bronchial tubes and the lungs

Bulbous—swollen and rounded in shape
Cardiomyopathy—disease of the heart muscle
CAS Registration Number—number assigned by the Chemical Abstracts Service to identify a chemical
Central nervous system (CNS)—portion of nervous system consisting of the brain and spinal cord
Cholinergic—stimulated by or releasing acetylcholine or a related compound
Cholinesterase—enzyme that hydrolyzes acetylcholine into choline and acetic acid and is important in the functioning of the nervous system
Chromatid—one of the two spiral filaments making up the chromosome
Chromosomes—structures in the cell nucleus that contain DNA
Chronic effect—biological change persisting over a major portion of a lifetime
Chronic exposure—exposure (usually low-level) during a major portion of lifetime to an environmental alteration that may or may not cause a health problem; used by CERCLA to describe an exposure period for humans of 7 yr or more
Clonic—pertaining to alternate muscular contraction and relaxation in rapid succession
Conjunctivitis—inflammation of the lining of the eyelids
Cornea—five-layered transparent tunic over the anterior portion of the eye
Corpus luteum—yellow glandular mass in the ovary formed by the ovarian follicle
Cretinism—arrested physical and mental development resulting from lack of secretions from thyroid
Ct—concentration of chemical in air multiplied by time of exposure
Diestrus—short period of sexual quiescence between metestrus and proestrus
Dysarthria—impaired speech caused by damage to the nervous system
Dyspnea—difficult or labored breathing
Edema—presence of abnormally large amounts of fluid in intercellular spaces of body tissues
Emesis—vomiting
Encephalocele—hernia of the brain
Endpoint—biological effect used as an index of the effect of a chemical on an organism
Enteritis—inflammation of the intestine
Epididymis—testicular structure in which spermatozoa are stored
Epithelium—cells covering the internal and external surfaces of the body
Erythema—redness of the skin produced by congestion of the capillaries
Estimated Reference Dose (RfD_e)—toxicity value calculated in this report from the available toxicological data; but not yet verified by EPA (see Reference Dose)
Exencephaly—abnormality in which the brain develops outside the skull
Fasciculations—small local contractions of muscles
Fetotoxic—toxic to developing embryos

Gavage—forced feeding, particularly with a tube passed into the esophagus or stomach
Gravid—containing eggs
Health Advisory—estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials
Hemangioendothelioma—benign tumor composed of new formed blood vessels in which the endothelial cells are the most prominent component
Hematocrit—ratio of the volume of packed red blood cells to the volume of whole blood
Hematopoiesis—process by which blood and blood cells are formed
Hematuria—blood in the urine
Hemoglobin—oxygen-carrying pigment present in red blood cells
Hepatoma—malignant tumor occurring in the liver
Heterozygous—possessing different alleles at a given locus
Hippocampus—region of the limbic system of the brain that is involved with memory of context and emotional meaning
Homeostasis—relatively stable state of chemical equilibrium
Homozygous—possessing two identical alleles of a gene
Human Equivalent Dose—dose that is expected to produce in humans the same effect as that observed in laboratory animals
Hydroureter—abnormal distension of the ureter with urine or watery fluid
Hypalgesia—diminished sensitiveness to pain
Hyperglycemia—excess of sugar in the blood
Hyperplasia—abnormal increase in cell number
Hypoxia—low oxygen content or tension; deficiency of oxygen in the inspired air
Intrauterine—within the uterus
Keratitis—inflammation of the cornea of the eye
Keratoacanthoma—rapidly growing papular skin lesion with a crater filled with a keratin plug
Lacrimation—secretion and discharge of tears
LC_{50}—statistically derived concentration of a chemical in air or water that is expected to cause death in 50% of test animals; median lethal concentration
LC_{Lo}—lowest concentration of a chemical in air or water resulting in a lethal effect
LD_{50}—statistically derived dose of a chemical expected to cause death in 50% of the test animals; median lethal dose, usually expressed as mg/kg body weight
LD_{Lo}—lowest dose that causes a lethal effect
Lesion—pathological or traumatic discontinuity of tissue or loss of function of a body part
Leukemia—disease characterized by the proliferation of white blood cells
Leukopenia—reduction in the number of white blood cells

LOAEL—lowest-observed-adverse-effect level; the lowest dose producing an observable adverse effect
Lymphosarcoma—any malignant neoplastic disorder of lymphoid tissue, excluding Hodgkin's disease
Mesenteric—associated with the mesenteries; i.e., double-folded peritoneal membranes connecting the intestines with the dorsal wall of the abdominal cavity
Metestrus—period of subsiding follicular activity following estrus
Microphthalmia—abnormal smallness of the eyes
Miosis (or myosis)—contraction of the pupil of the eye
Modifying Factor—adjustment factor used in RfD derivation, greater than 0 and less than or equal to 10; its magnitude reflects professional judgment regarding aspects of the data used for the assessment; e.g., the completeness of the overall data base and the number of animals tested
Muscarinic—pertaining to neural functions mediated by muscarinic cholinergic receptors (in the parasympathetic system and CNS)
Myelocytic leukemia—abnormal malignant proliferation of immature leukocytes
Myelopoiesis—formation of cells in the bone marrow
Myocardial—pertaining to the muscular tissue of the heart
Neoplasm—new growth of tissue serving no physiological function; benign, potentially malignant, or malignant
Nephritis—inflammation of the kidney
Neuropathy—functional disturbances and/or pathological changes in the peripheral nervous system
Neurotoxicity—exerting a destructive or poisonous effect on nerve tissue
NOAEL—no-observed-adverse-effect level; the highest dose in an experiment that did not produce an observable adverse effect
Ocular—pertaining to or affecting the eye
Oropharynx—section of the pharynx between the soft palate and the upper edge of the epiglottis
Papilloma—branching or lobulated benign tumor derived from epithelial tissue
Parasympathetic—craniosacral portion of the autonomic nervous system
Parenteral—introduced other than by way of the intestines, (e.g., subcutaneous, intramuscular, intravenous)
Phosphorylation—process of introducing the trivalent PO group into an organic molecule
Pneumonitis—inflammation of the lungs
Petechial—small purple spots on skin, mucous membranes, or internal organs
Photophobia—abnormal visual intolerance to light
Precordial—region over the heart and lower part of the thorax
Proestrus—period of heightened follicular activity preceding estrus
Proteinuria—excess of serum proteins in the urine; also called albuminuria
Psychomotor—pertaining to the motor effects of cerebral activity
Pulmonary—pertaining to the lungs

Reference Dose (RfD)—an estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effect during a lifetime; RfD is expressed in units of milligrams of chemical per kilogram body weight per day

Renal—pertaining to the kidney

Retrobulbar—behind the eyeball

Rhabdomyosarcoma—highly malignant tumor of striated muscle

Rhinitis—inflammation of the mucous membranes of the nose

Sensitization—initial exposure to a specific antigen resulting in an immune response; subsequent exposures result in stronger immune responses

Serum—clear, watery fluid that moistens the surface of internal membranes; the watery portion of blood which remains after the blood clots

Slope factor—slope of the upper-bound dose extrapolation model for carcinogenicity at doses approaching zero

Spermatogenesis—process of formation of mature male germ cells

Subchronic—of intermediate duration; used by CERCLA to describe studies or levels of exposure for humans between 2 wk and 7 yr

Sympathetic—autonomic nervous system, which controls smooth muscles, blood vessel diameter, and glandular secretion

Synapse—gap between nerve axons, or between axons and effector organs

Syndactylous—fusion of adjacent toes

Systemic—pertaining to or affecting the body or organism as a whole

Systemic effects—effects requiring absorption and distribution of a toxicant to a site within the body distant from its entry point

Tachycardia—excessively rapid heartbeat

Temporal—relating or pertaining to time

Teratogenesis—induction of structural or functional development abnormalities by exogenous factors acting during gestation; interference with normal embryonic development

Teratogenic—tending to produce anomalies of formation or development during gestation

Teratogenicity—capacity of a physical or chemical agent to cause nonhereditary congenital malformations (birth defects) in offspring

Time-weighted average—average value of a parameter (e.g., concentration of a chemical in air) that varies over time

Tonic convulsions—prolonged contraction of muscles

Torticollis—contracted state of the cervical muscles, resulting in a twisted neck

Tracheitis—inflammation of the trachea

Ulceration—sloughing of inflamed and necrotic tissue

Uncertainty Factors—numeric adjustments used to derive an RfD from experimental data. UFs are intended to account for the variation in sensitivity among the members of the human population (UF_H); the uncertainty in extrapolating animal data to humans (UF_A); the uncertainty in extrapolating

from data obtained in a study that is of less-than-lifetime exposure (UF_S); and the uncertainty in using LOAEL data rather than NOAEL data (UF_L)

Urticaria—vascular reaction of the skin marked by the transient appearance of smooth, slightly elevated patches (wheals) that are redder or paler than the surrounding skin and often attended by severe itching

Vascular—pertaining to blood vessels

Vertigo—dizziness

Vesicant—blister-causing chemical agent

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